Newborn Screening Quality Assurance Program

2001 ANNUAL SUMMARY REPORT

Volume 19 April 2002

INTRODUCTION

The Newborn Screening Quality Assurance Program (NSQAP) is designed to help screening laboratories achieve excellent technical proficiency and maintain confidence in their performance while processing large volumes of specimens daily. We continually strive to produce certified dried-blood spot (DBS) materials for reference and quality control (QC) analysis, to improve the quality and scope of our services, and to provide immediate consultative assistance. Through our interactive efforts with the program's participants, we aspire to meet their growing and changing needs. We always welcome comments and suggestions on how we may better serve the newborn screening laboratories.

A major public health responsibility, newborn screening for detection of treatable, inherited metabolic diseases is a system consisting of six parts: education, screening, follow-up, diagnosis, management, and treatment. Effective screening of newborns using DBS specimens collected at birth, combined with follow-up diagnostic studies and treatment, helps prevent mental retardation and premature death. These blood specimens are routinely collected from more than 95% of all newborns in the United States. State public health laboratories or their associated laboratories routinely screen DBS specimens for inborn errors of metabolism and other disorders that require intervention. For more than 23 years, the Centers for Disease Control and Prevention (CDC), with its cosponsor, the Association of Public Health Laboratories (APHL), has conducted research on materials development and assisted laboratories with quality assurance (QA) for these DBS screening tests. The QA services primarily support newborn screening tests performed by state laboratories; however, we also accept other laboratories and international participants into the QA program. All laboratories in the United States that test DBS specimens participate voluntarily in NSQAP. Currently, the program provides QA services for congenital hypothyroidism, phenylketonuria, galactosemia, congenital adrenal hyperplasia, maple syrup urine disease, homocystinuria, biotinidase deficiency, galactose-1-uridyltransferase (GALT) deficiency, and hemoglobinopathies.

The QA program consists of two DBS distribution components: QC materials for periodic use and quarterly proficiency testing (PT). The QC program enables laboratories to achieve high levels of technical proficiency and continuity that transcend changes in commercial assay reagents while maintaining the high-volume specimen throughput that is required. The QC materials, which are intended to supplement the participants' method- or kit-control materials, allow participants to monitor the long-term stability of their assays. The PT program provides laboratories with quarterly panels of blind-coded DBS specimens and gives each laboratory an independent external assessment of its performance. DBS materials for QC and PT are certified for homogeneity, accuracy, stability, and suitability for all kits manufactured by different commercial sources.

Over the last six years, NSQAP has grown substantially, both in the number of participants and in the scope of global participation (Figure 1). In 2001, 256 laboratories in 47 countries (at least one laboratory per country) were active program participants; of these, 186 participated in the PT component and 176 in the QC part (Figure 2). DBS materials for ten analytes were distributed to participating laboratories (Figure 3). For biotinidase, galactose-1-phosphate uridyltransferase (GALT), and hemoglobins, QC materials were not distributed because of the limited availability of appropriate blood sources.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention (CDC)
and the
Association of Public Health Laboratories

NSQAP
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Program Information Web site:
http://www.cdc.gov/nceh/dls/newborn_screening.htm

Data-reporting Web site:
http://www2.cdc.gov/nceh/NewbornScreening

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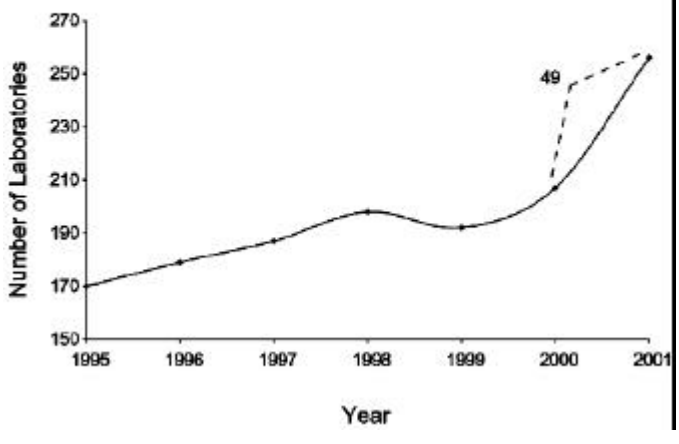
NEW ACTIVITIES

In 2001, NSQAP operated a pilot PT program for laboratories testing DBS by tandem mass spectrometry (MS/MS) for detection of amino acid metabolic disorders, urea cycle disorders, fatty acid oxidation disorders, and organic acid metabolic disorders. During the year, the program distributed three MS/MS PT panels; data from these panels are not included in this report but are available in other QA reports by request. We plan to bring the MS/MS component into a PT evaluation status in 2002 with the application of cutoff decisions and presumptive classifications for grading. A separate summary report for the 2001 MS/MS data from the pilot phase is planned for 2002; however, some MS/MS data are available for amino acid disorders in this annual report. A pilot PT program is under development to serve those laboratories screening newborns for biomarkers of cystic fibrosis. In 2002, we plan to distribute panels of DBS for immunoreactive trypsinogen (IRT) measurements in a pilot PT program format and to pursue sources of DBS materials for the DNA testing component, Δ508 mutation. The addition of these PT and QC efforts to the NSQAP will expand QA coverage to more than 30 newborn disorders. To support this increase, we purchased a custom-built robotic system for producing DBS QC materials. Using this system greatly enhances our production capacity while requiring fewer staff to operate.

FIGURE 1. Laboratory Participation in the Newborn Screening Quality Assurance Program, 1995-2001

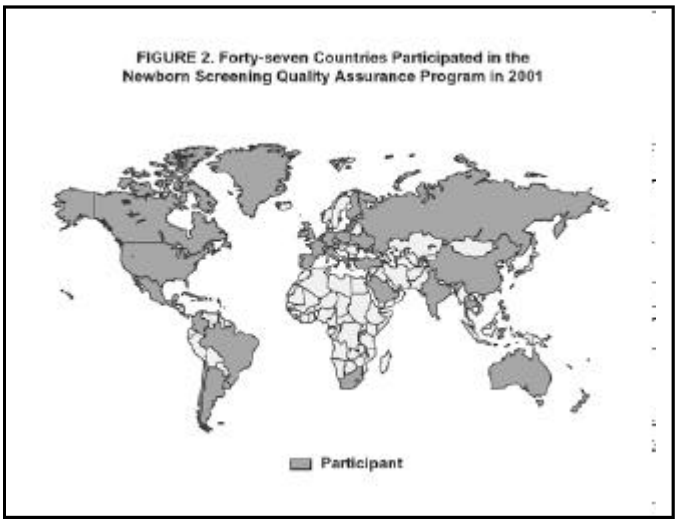


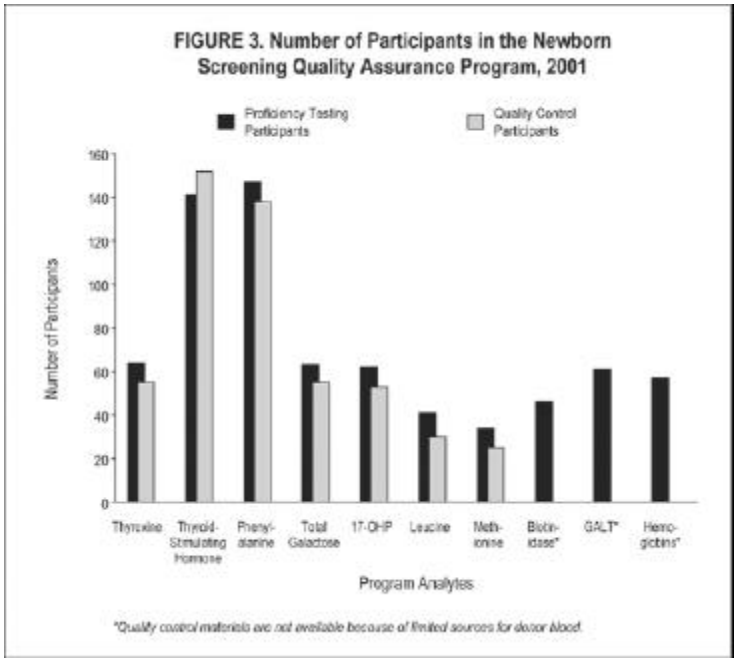
The NSQAP cosponsored and helped organize the second MS/MS meeting, “Enhancing the Implementation of Tandem Mass Spectrometry for Newborn Screening Laboratories,” on September 10-11, 2001, in Madison, Wisconsin. This meeting was designed (1) to bring

together a core discussion group of laboratory and medical scientists with a vested interest in successful newborn screening and with differing levels of expertise and experience using MS/MS technology and (2) to address solutions to problems encountered with implementation of MS/MS testing. The meeting of approximately 200 participants was successful. Conference proceedings will be published in 2002.

In 2001, APHL organized a subcommittee of the Newborn Screening and Genetics in Public Health Committee for quality assurance/quality control/ proficiency testing. One mission component of this subcommittee is to provide guidance to the NSQAP on procedures, policies, and activities for the quality assessment of laboratory testing. In January 2002, this subcommittee held its inaugural meeting in Atlanta, where the staff of the NSQAP provided an overall review of their activities. We believe that input from this subcommittee will enhance our continuing efforts to better serve our participants.

In January 2002, after months of programming and testing, NSQAP officially went "online" with the operation of its paperless data-reporting system whereby global participants can report quarterly PT data over the Internet. In addition, quarterly PT reports for inborn errors of metabolism, biotinidase deficiency, and GALT deficiency panels can be viewed online by participants with user-specific IDs and passwords.The summary data for each quarter beginning in 2002 are available for public view at http://www2.cdc.gov/nceh/NewbornScreening. The PT programs for hemoglobinopathies and MS/MS are not as yet online but are scheduled as future enhancements.

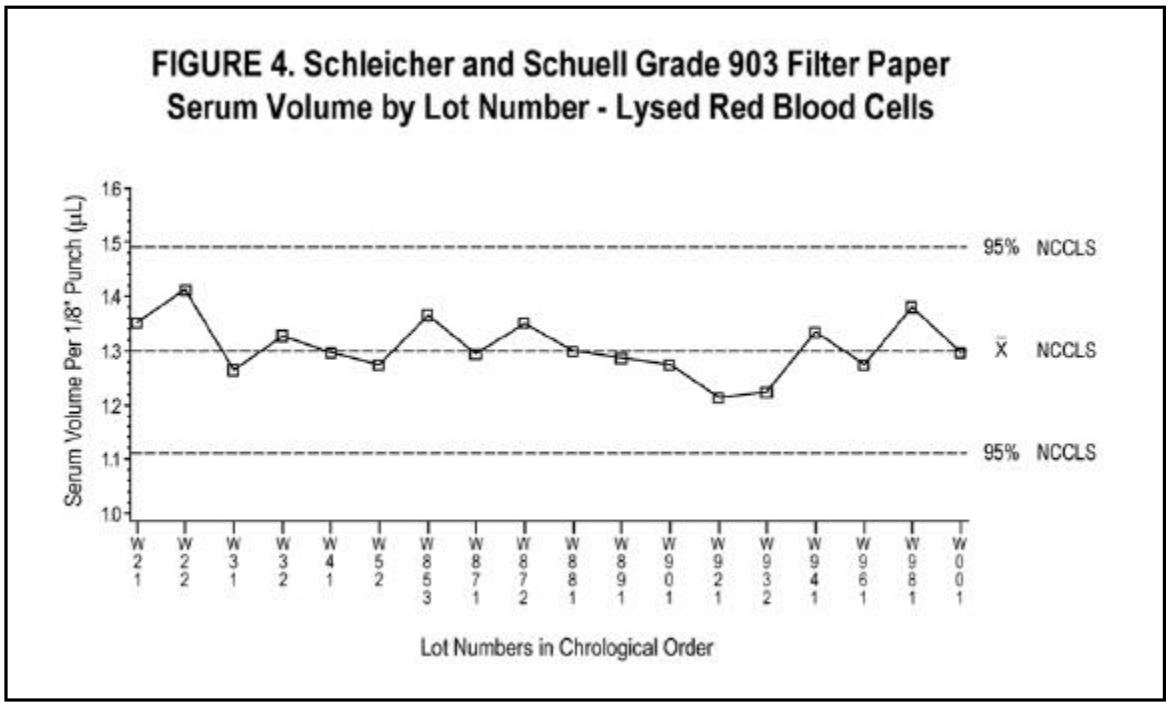


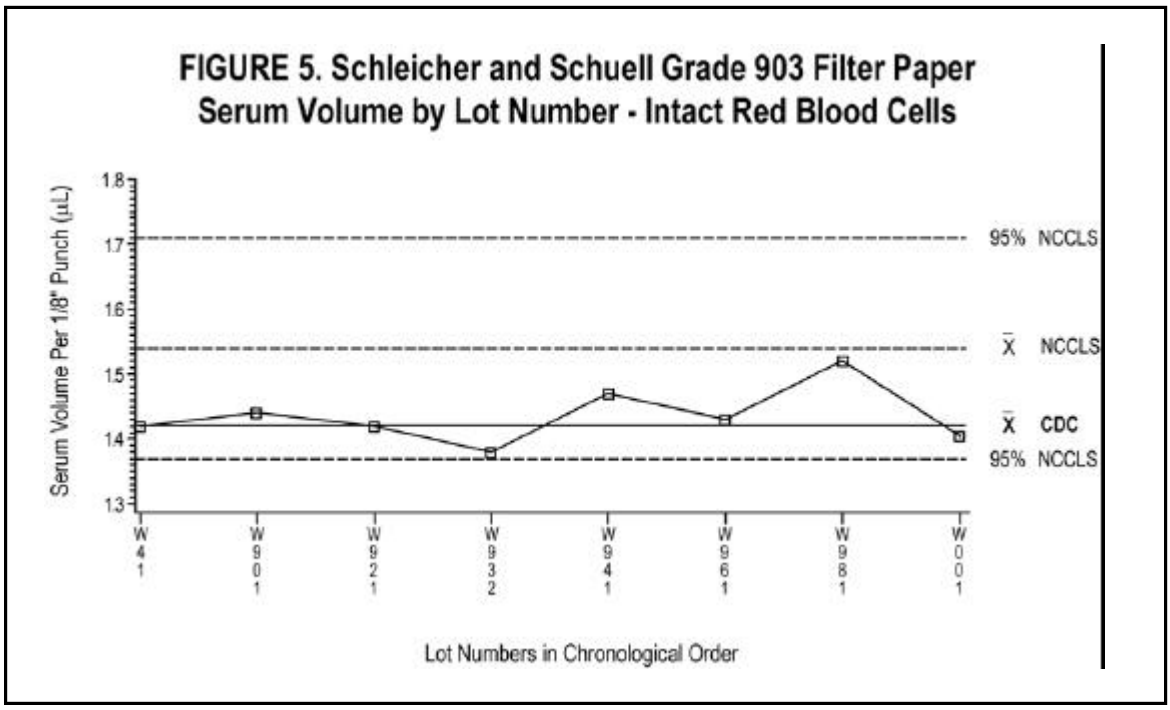


FILTER PAPER

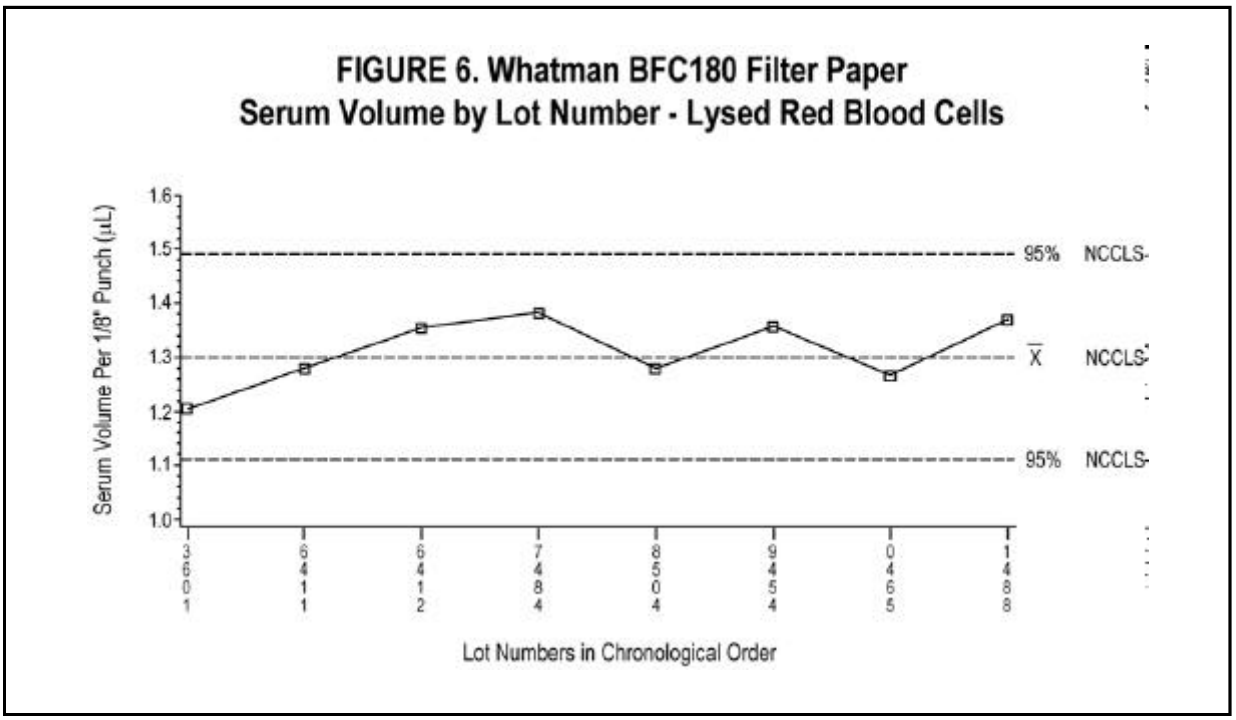
The paper disk punched to aliquot DBS specimens is a volumetric measurement and requires a degree of uniformity among and within production lots. As part of the QA program, we used an isotopic method[1] developed at CDC to evaluate and compare different lots of filter paper. Mean counts per minute of added isotopic-labeled T_4 within a 1/8-inch disk were equated with the serum volume of the disks from the dried whole blood specimens. In comparing production lots, we used statistical analyses of the counting data to determine values for homogeneity and serum absorption of the disks. To avoid the variability contributed by uncontrolled red blood cell (RBC) lysis, we initially used lysed-cell whole blood for variance studies with filter paper. The results of later studies have indicated that RBC lysis during the process is not sufficient to contribute substantially to the variance; however, the mean serum volume per disk is different with intact-cell blood.

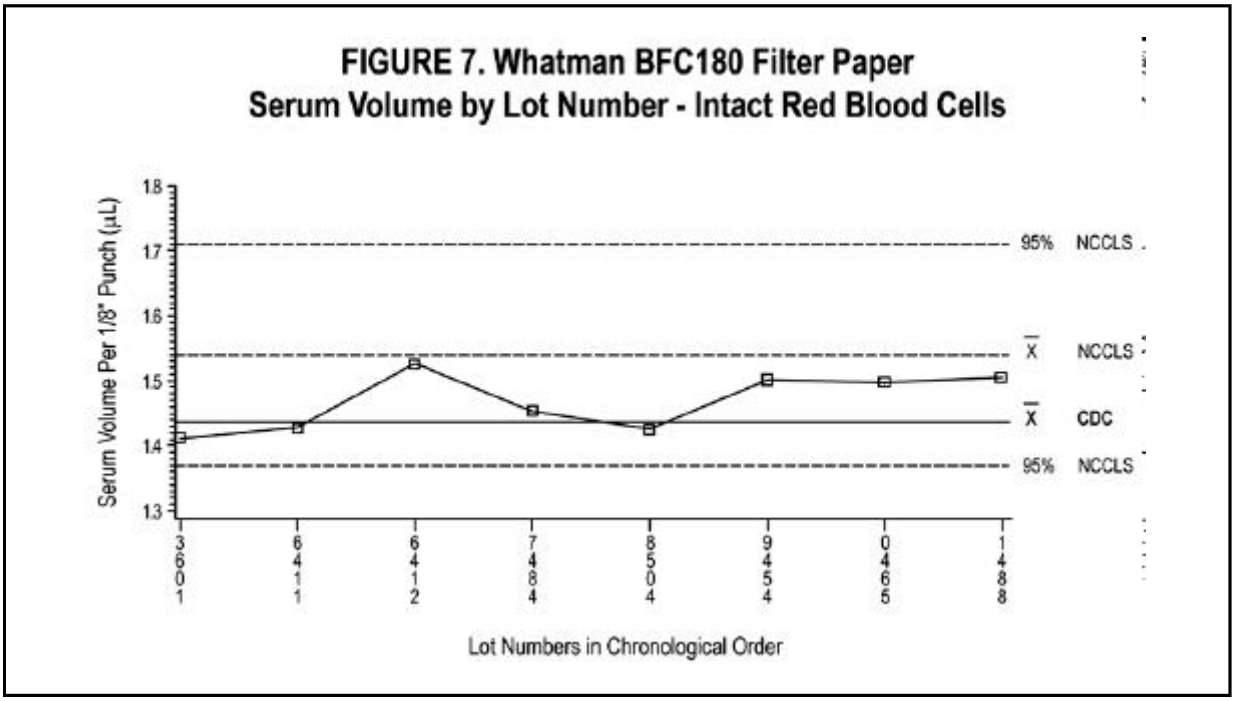
A pilot PT program is under development to serve the laboratories screening newborns for cystic fibrosis markers.





For historical reference and for maintaining uniformity of testing on all the paper production lots, we have continued using the lysed-cell procedure. We also measure performance with intact-cell preparations. The published and standardized acceptable volumes per 1/8-inch disk are 1.30 ± 0.19 µL (mean value and 95% confidence interval) for lysed-cell blood and 1.54 ± 0.17 µL for intact-cell blood.[1] As shown in Figures 4-7, the mean values and confidence intervals (CI) are the filter-paper evaluation parameters published in the NCCLS approved standard.[1] As shown in Figures 5 and 7, the second mean value (solid line) is the mean value produced from the NSQAP database. This year, the line was added for reference. The mean values for all lots are within the 95% CI defined by NCCLS but are below the mean values indicated by the NCCLS standard.[1] The mean value and CI for the intact cell measurements will be examined and discussed during the routinely scheduled review period for the NCCLS standard in 2002. The original published values were not produced at CDC. Only recently have we accumulated sufficient data for intact cell measurements to review the CI assessments for different filter paper lots.





Filter paper lots used in the CDC production of QC and PT specimens distributed in 2001 were W961 and W981 of Grade 903. All filter paper lots were analyzed for agreement with the evaluation parameters according to the NCCLS approved standard.[1]

Each year, with the extensive cooperation of manufacturers (Schleicher & Schuell and Whatman) of filter papers approved by the Food and Drug Administration (FDA) for blood collection, we have conducted routine evaluations of new lots and compared new lots with previous lots. The criteria for acceptable performance are the approved limits established in the NCCLS standard.[1] Each manufacturer is also expected to establish its own testing program using the NCCLS standard and make available to the user its certification data for each distributed lot of paper. The independent evaluations by CDC are an impartial and voluntary service offered as a function of our quality assurance program and do not constitute preferential endorsement of any product over other specimen collection papers approved by the FDA.

The serum-absorbance volumes of 18 lots of Grade 903 filter paper (Schleicher & Schuell, Keene, NH) determined from lysed-RBC blood and for 8 lots determined from intact-RBC blood, are shown in chronological order. For W001, the most recent production lot of Grade 903 filter paper, we found the mean serum-absorbance volume to be 1.30 µL for a 1/8-inch disk for lysed-cell blood and 1.40 µL per 1/8-inch disk for intact-cell blood. Each mean value is within the acceptable range for the matrix used. Lot W001 was homogeneous (i.e., the measured within-spot, within-sheet, and among-sheets variances were within the acceptable limits).

In 1996, the FDA approved the filter paper, BFC180, produced by Whatman Inc. (Fairfield, NJ) as a blood collection device. The BFC180 was evaluated by CDC according to the criteria previously described.[1] The serum-absorbance volumes for eight lots of BFC180 filter paper determined from lysed-RBC blood and determined from intact-RBC blood, are shown in chronological order. For 1488, the most recent production lot of BFC180 filter paper, we found the mean serum-absorbance volume to be 1.37 µL for a 1/8-inch disk for lysed-cell blood and 1.51 µL per 1/8-inch disk for intact-cell blood. Each mean value is within the acceptable range for the matrix used. Lot 1488 was homogeneous (i.e., the measured within-spot, within-sheet, and among-sheets variances were within the acceptable limits).

For the most recent lots of filter paper, W001 of Grade 903 and 1488 of BFC180, the mean serum-absorbance volumes are 1.40 and 1.51, respectively, per 1/8-inch disk for intact-cell blood.

SPECIMEN PREPARATION AND DATA HANDLING

Tables and figures show the enriched concentrations of all PT specimens and QC lots as well as the summarized quantitative data. The total concentration of each specimen or lot was equal to the sum of the enriched concentration and the endogenous concentration (nonenriched). For T_4 PT specimens, the CDC assayed values were reported because of differences in the blood sources used for DBS production. Some specimens were enriched above the endogenous T_4 concentration, and some were enriched with T_4 after T_4 depletion of the base serum. Except for biotinidase and GALT, all DBS specimens in the PT surveys and QC production lots were prepared from whole blood of 55% hematocrit. Purified analytes or natural donor blood, except for TSH, which used the Second International Reference Preparation (80/558), were used for all enrichments. For galactosemia, enrichments were made with galactose, galactose-1-phosphate, or both so that both free galactose (galactose alone) and total galactose (free galactose plus galactose present as galactose-1-phosphate) could be measured. For biotinidase and GALT, individual donor blood with hematocrit, adjusted to 50%, was used. All reported analytic values outside the 99% confidence limits were excluded from the summaries of quantitative results.

For obtaining data on the QC materials, we estimated the method response to endogenous materials by performing weighted linear regression analyses for mean-reported concentrations versus enriched concentrations. We then extrapolated the regression lines to the Y-axis to obtain an estimate of the observed endogenous analyte concentration for each method category. These estimates are reliable when (1) enrichments are accurate, (2) the analytic method gives a linear response across the range of the measurements, and (3) the slopes for regression lines are approximately equal to one.

In 2001, we applied the laboratory-reported specific cutoff values, when available, to our judgment algorithm for clinical assessments; otherwise, we used the NSQAP-assigned working cutoff values that are based on the national mean value for this assessment.

CUTOFFS

When reporting cutoff values, we requested the first decision level for sorting test results that are reported as presumptive positive (outside limits) from results reported as negative (within limits). The cutoff values shown in Figures 8a-8c illustrate the distribution of reported cutoffs for domestic and foreign laboratories. The values for the mean (arithmetic average) and the mode (most frequent value) are shown for each analyte. The mean cutoff values for domestic and foreign laboratories were similar except those for 17-OHP, which were twice as high for domestic laboratories. The cutoff values for Phe and TSH for both domestic and foreign laboratories show a large scatter around the mean value. This observation is somewhat surprising because Phe and TSH are the most common and historical analytes in newborn screening. For domestic laboratories, the Phe mean and mode values are the same. The scatter of cutoff values for total galactose is larger for foreign laboratories than for domestic laboratories.

When reporting cutoff values, we requested the first decision level for sorting test results that are reported as presumptive positive (outside limits) from results reported as negative (within limits).

FIGURE 8a. Cutoff Values for Domestic and Foreign Laboratories by Analyte

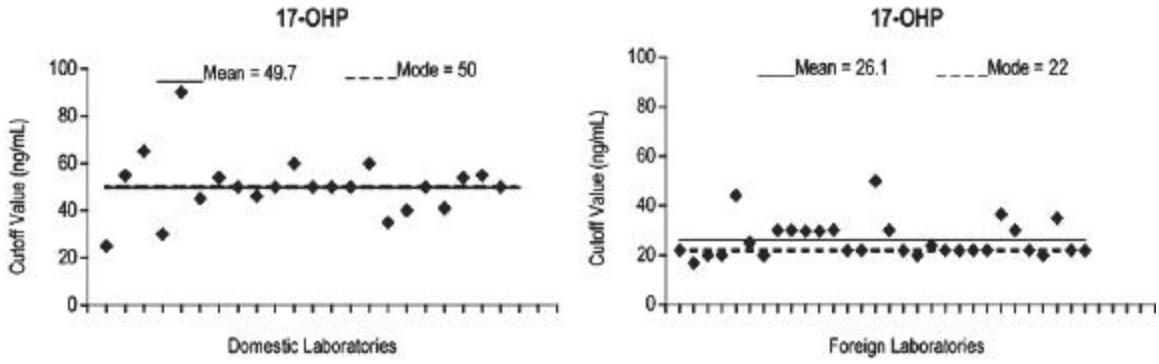


FIGURE 8b. Cutoff Values for Domestic and Foreign Laboratories by Analyte

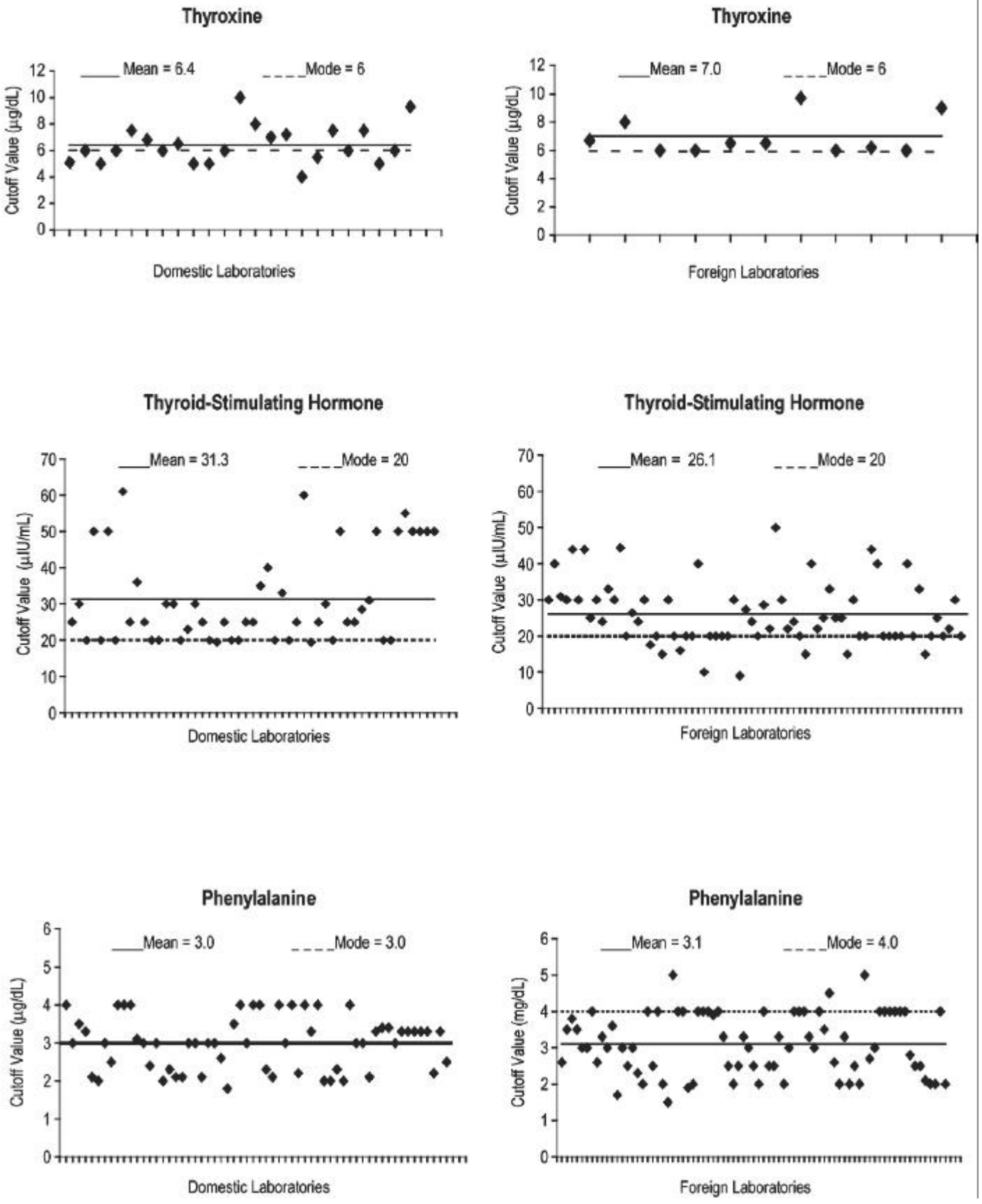
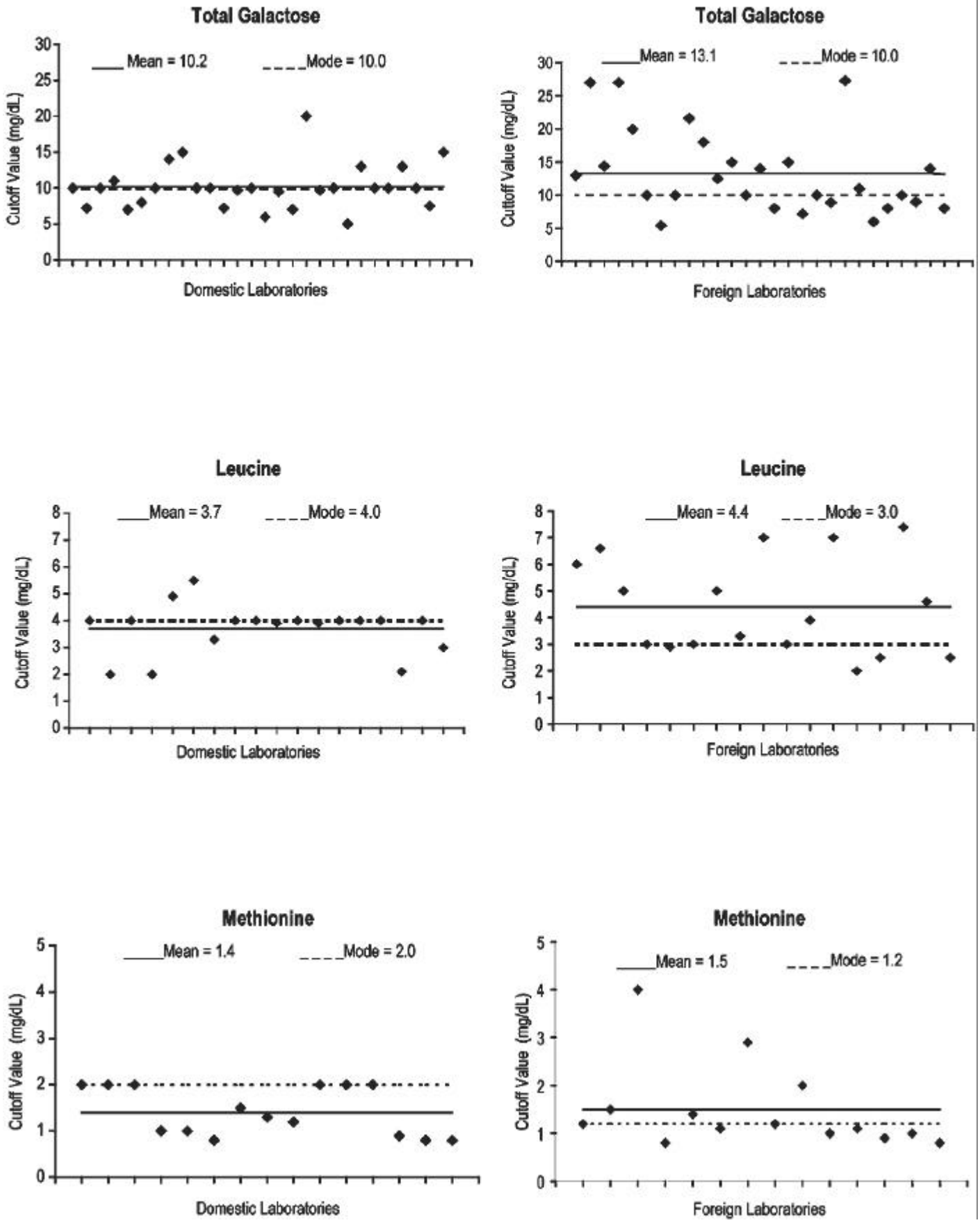


FIGURE 8c. Cutoff Values for Domestic and Foreign Laboratories by Analyte



PROFICIENCY TESTING

All PT panels contained five blind-coded 100-µL DBS specimens. Specimens in the PT panels contained either endogenous levels or were enriched with predetermined levels of thyroxine (T_4), thyroid-stimulating hormone (TSH), phenylalanine (Phe), total galactose (Gal), 17 α-hydroxyprogesterone (17-OHP), leucine (Leu), and methionine (Met). Special separate panels for biotinidase deficiency and for GALT deficiency were prepared with purchased blood from donors with enzyme deficiencies. Specimens for the hemoglobinopathies panel were prepared from umbilical cord blood.

TABLE 1. 2001 Summary of Performance Evaluation Errors by Domestic and Foreign Laboratories

Domestic	Positive Specimens Assayed (N)	False-Negative Errors (%)	Negative Specimens Assayed (N)	False-Positive Errors (%)
Hypothyroidism	404	0.7	404	1.2
Phenylketonuria	538	0.6	478	1.5
Galactosemia	268	0	158	0.6
Congential Adrenal Hyperplasia	202	1.0	180	1.7
Maple Syrup Urine Disease	153	0.7	165	2.4
Homocystinuria	153	3.3	108	0
Biotinidase Deficiency	97	2.1	239	0.4
GALT Deficiency	223	0.4	535	1.1
Foreign	**Positive Specimens Assayed (N)**	**False-Negative Errors (%)**	**Negative Specimens Assayed (N)**	**False-Positive Errors (%)**
Hypothyroidism	614	0.3	537	3.2
Phenylketonuria	678	0.9	683	2.0
Galactosemia	299	1.3	199	2.0
Congential Adrenal Hyperplasia	288	0.3	314	2.5
Maple Syrup Urine Disease	153	2.6	160	6.7
Homocystinuria	159	2.5	128	3.1
Biotinidase Deficiency	109	6.4	316	1.9
GALT Deficiency	75	0	220	5.9

Specimen sets were packaged in a zip-close metallized plastic bag with desiccant, instructions for analysis, and data-report forms. We prepared and distributed quarterly reports of all results that had been received by the cutoff dates. In this annual report, Figures 9a-9g for quantitative data summarize the data from all PT reports received during 2001. Only the qualitative assessments are reported for the PT surveys for (1) sickle cell disorders and other hemoglobinopathies, (2) biotinidase deficiency PT surveys, and (3) pilot PT surveys for GALT deficiency. Presumptive clinical classifications (qualitative assessments) of some specimens may differ by participant because of specific clinical assessment practices. If participants provided us with their cutoff values, we applied these cutoffs in our final appraisal of the error judgment.

The PT quantitative results (Figures 9a-9g) are grouped by kit or method to illustrate any method-related differences in analyte recoveries. Because some of the pools in a routine PT survey represent a unique donor specimen, differences in endogenous materials in the donor specimens may influence method-related differences. The T_4 and TSH results showed a reasonably consistent performance among the different methods, with two methods showing slightly higher values for some T_4 specimens in Quarters III and IV. For Phe, the reported results show reasonable variability among the methods, except for one method that shows higher values. The recoveries for Phe were good for most methods when both enrichment and endogenous concentrations were weighted in the assessment. The among-method comparisons of mean values for most methods appear reasonable for Gal and 17-OHP, except for 17-OHP in Quarters II and IV where two methods gave elevated values for specimens with the higher concentrations. One method for total galactose, which was from the same source that produced high values for Phe, produced values higher than those of other methods. The values reported for Leu show little variability, but one method for Met produced higher values than the other ones.

TABLE 2. Summary of Performance Evaluation Errors for Hemoglobinopathies by Domestic and Foreign Laboratories

Hemoglobinopathies	Domestic	Foreign
Specimens assayed	990	85
Phenotype errors	0.5%	4.7%
Clinical assessment errors	0.5%	3.5%

FIGURE 9a. 2001 Proficiency Testing Data
Mean Reported Concentrations of Thyroxine by Specimen Number

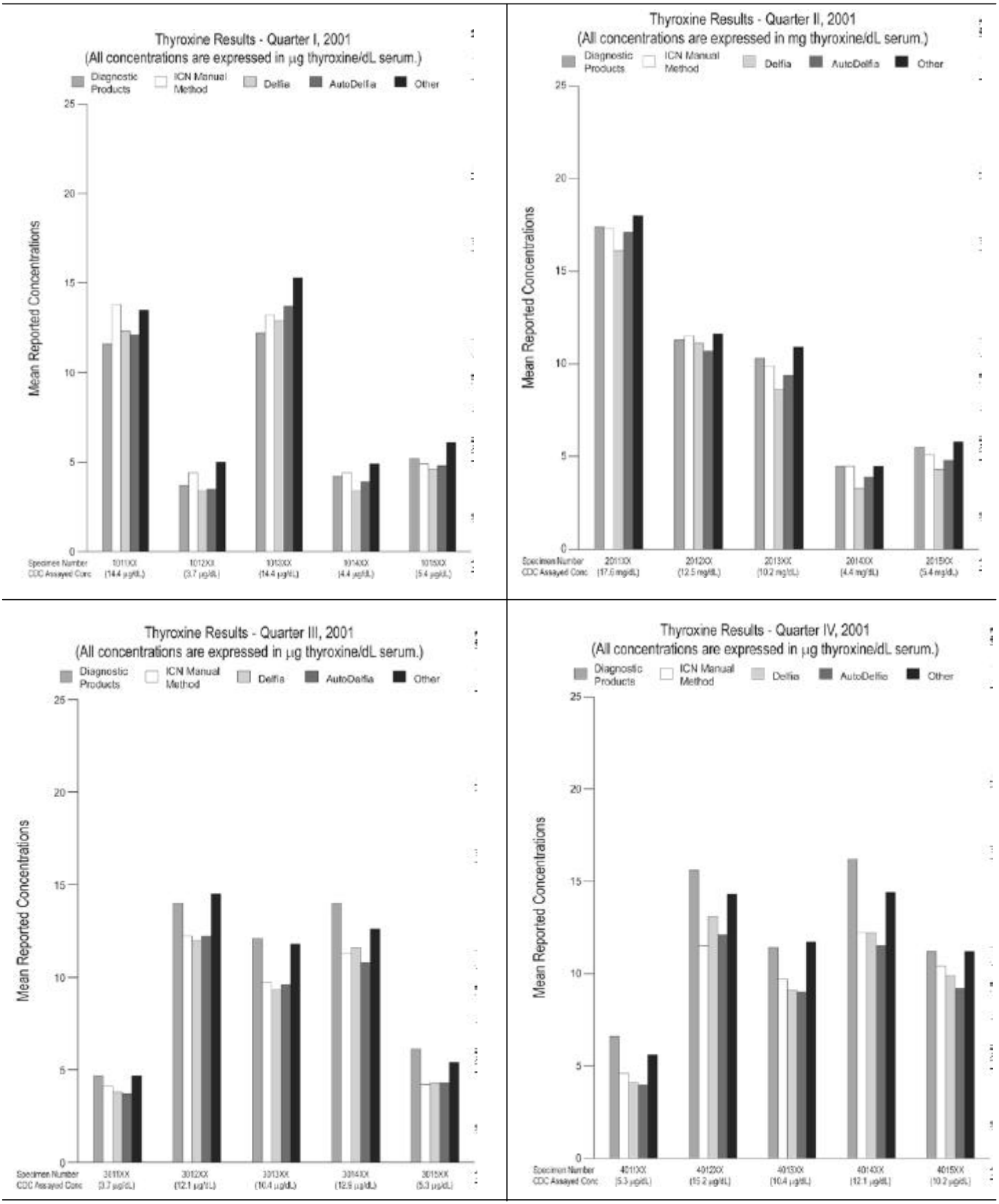
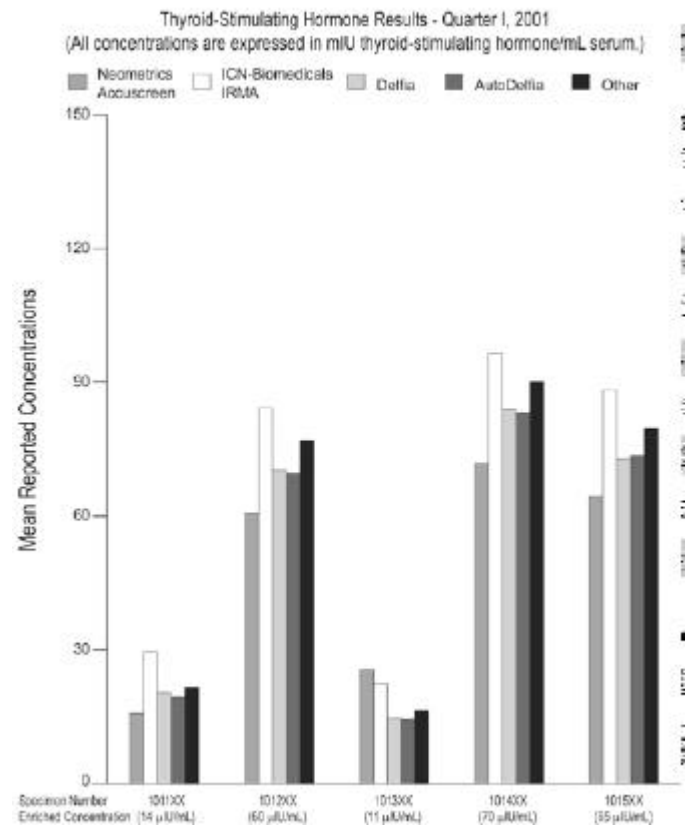
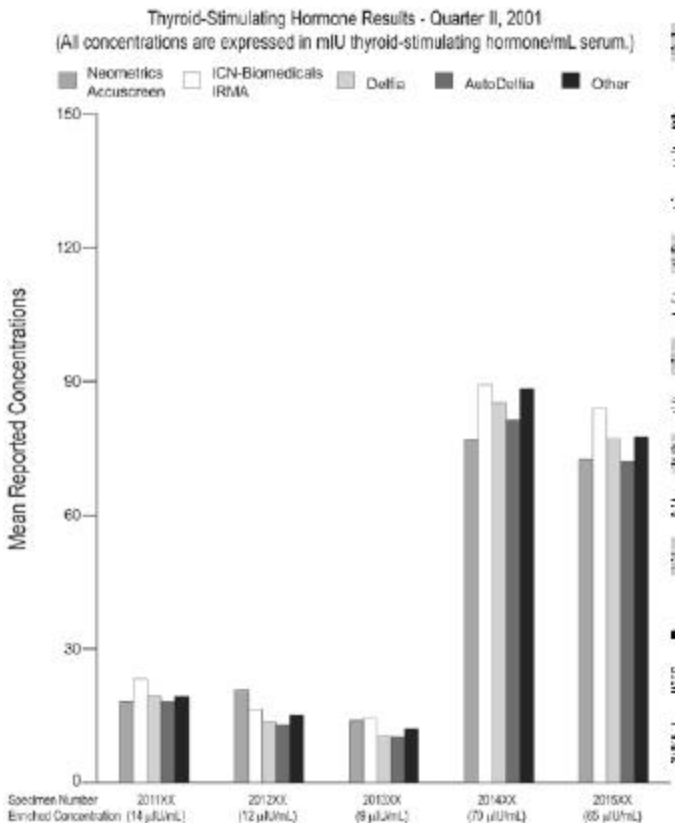
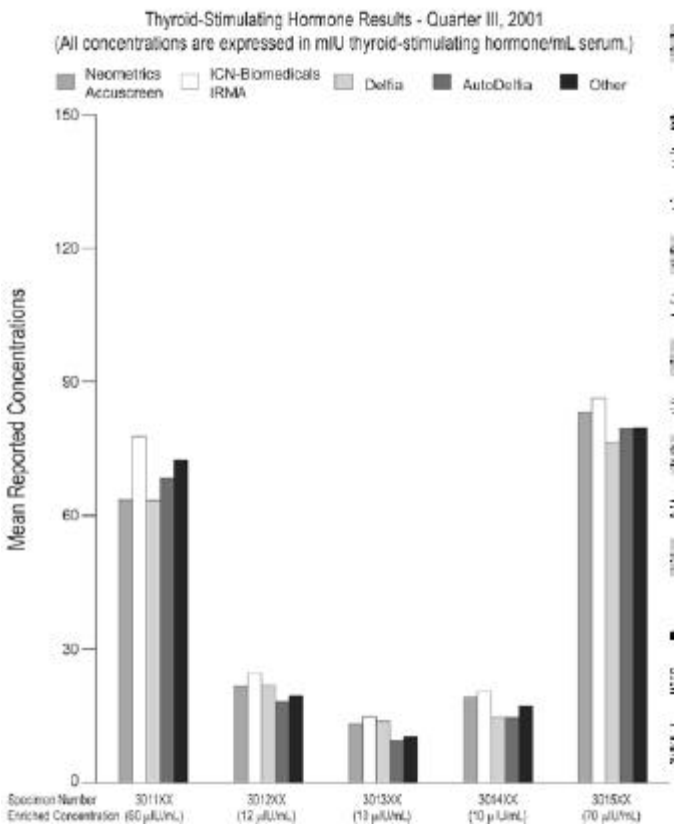


FIGURE 9b. 2001 Proficiency Testing Data
Mean Reported Concentrations of TSH By Specimen Number







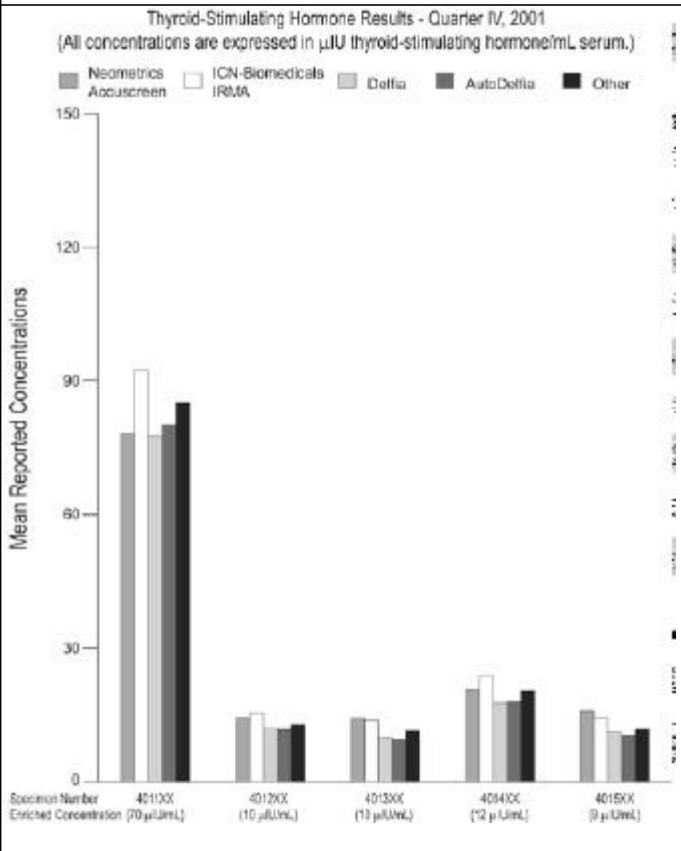
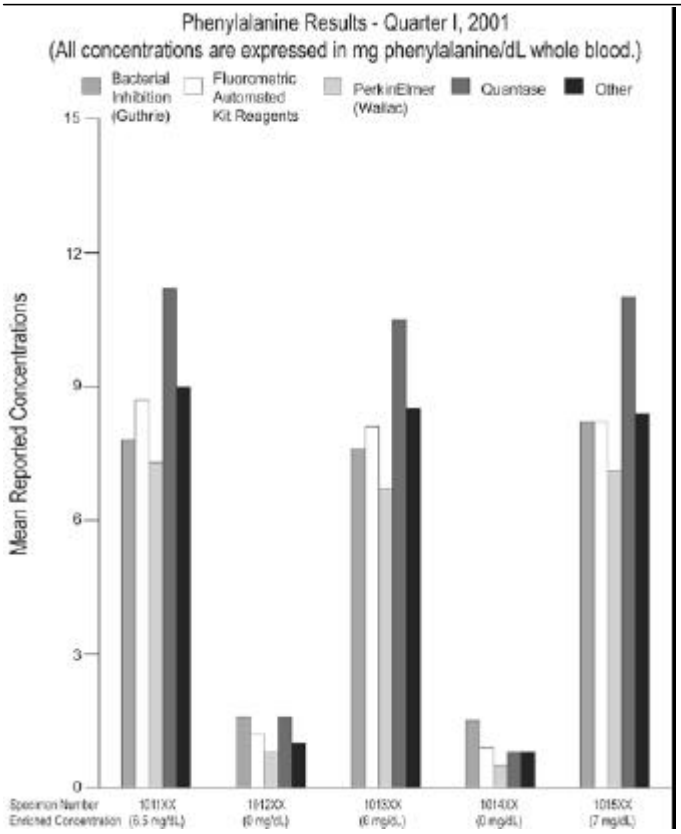
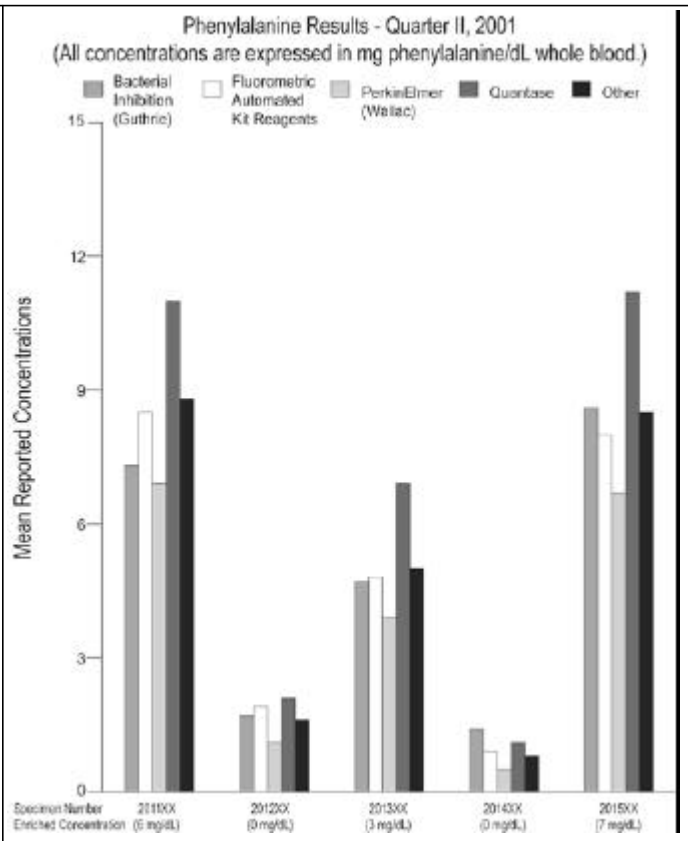
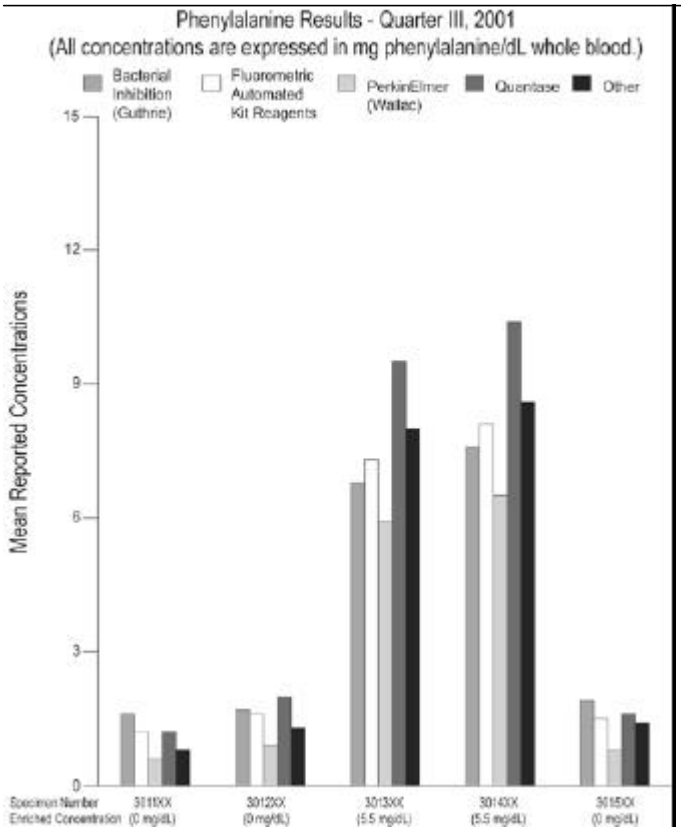


FIGURE 9c. 2001 Proficiency Testing Data
Mean Reported Concentrations of Phenylalanine By Specimen Number







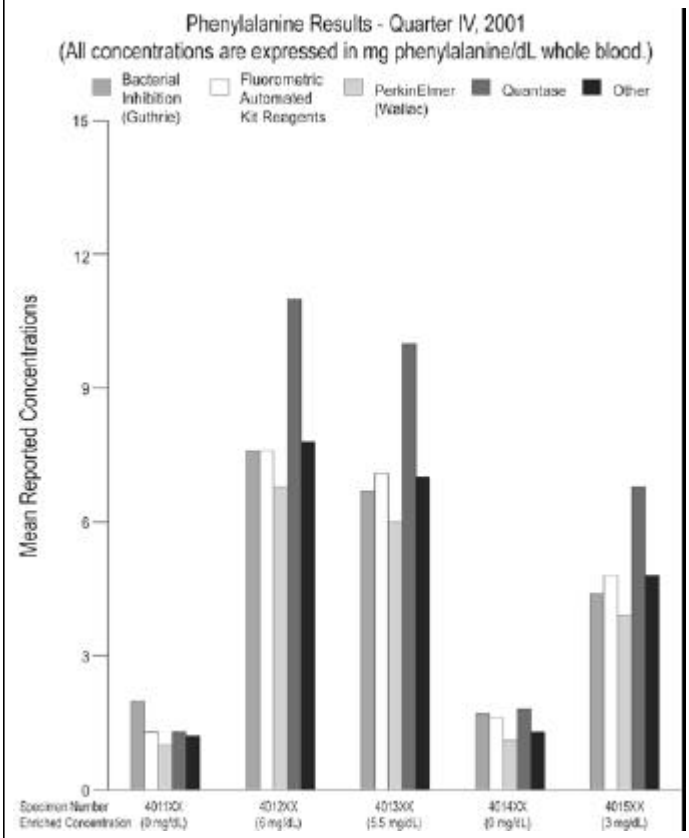
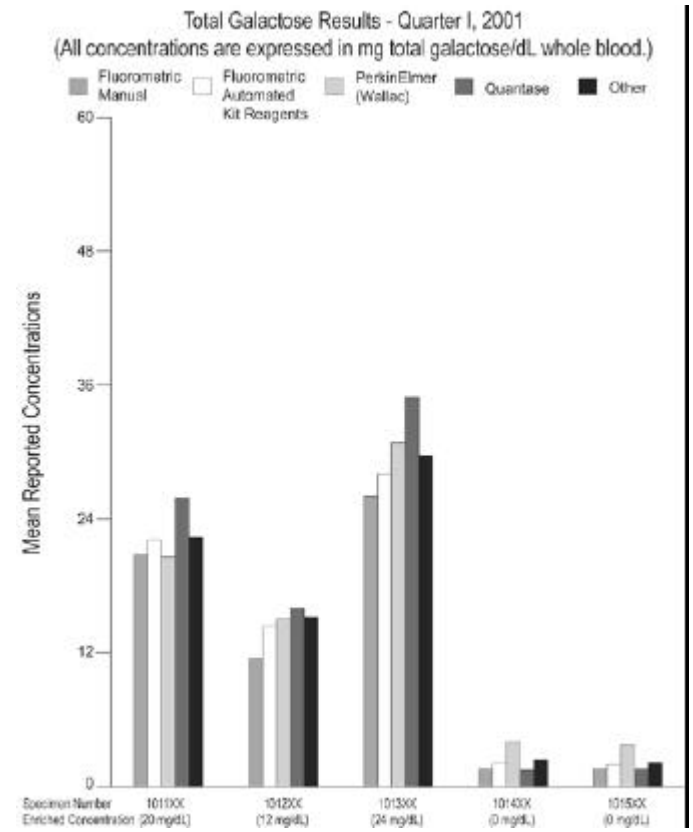
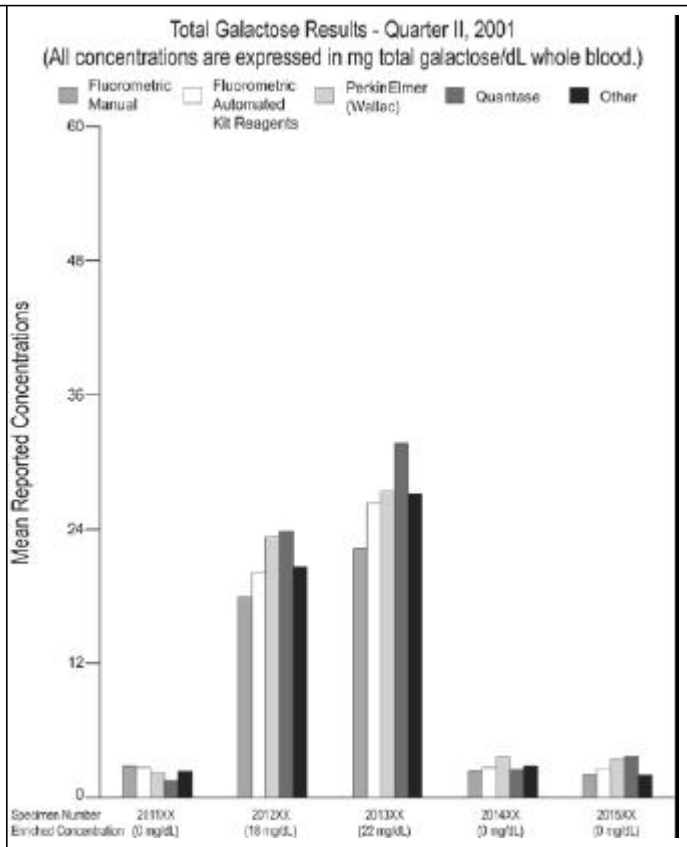
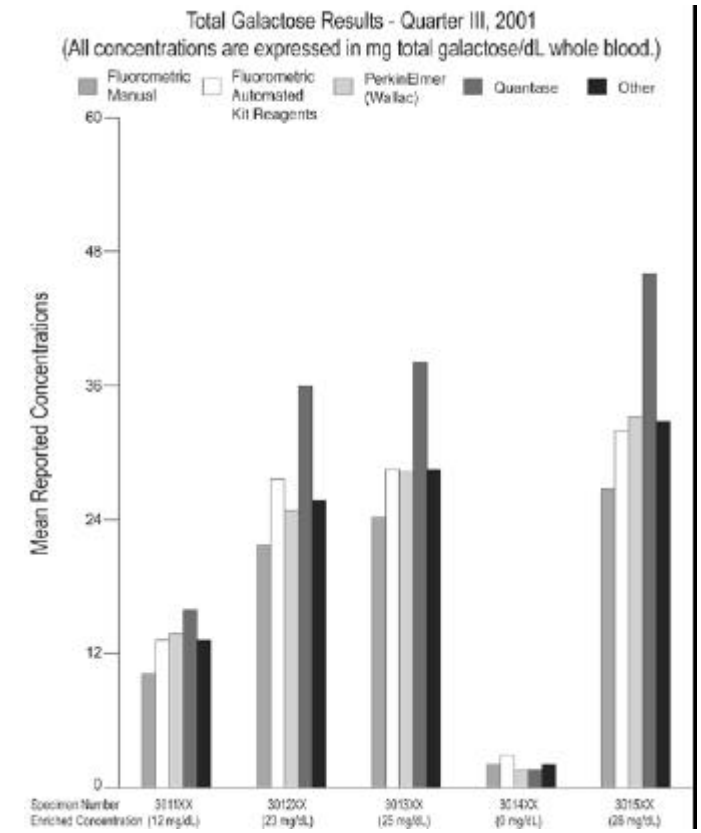


FIGURE 9d. 2001 Proficiency Testing Data
Mean Reported Concentrations of Total Galactose By Specimen Number







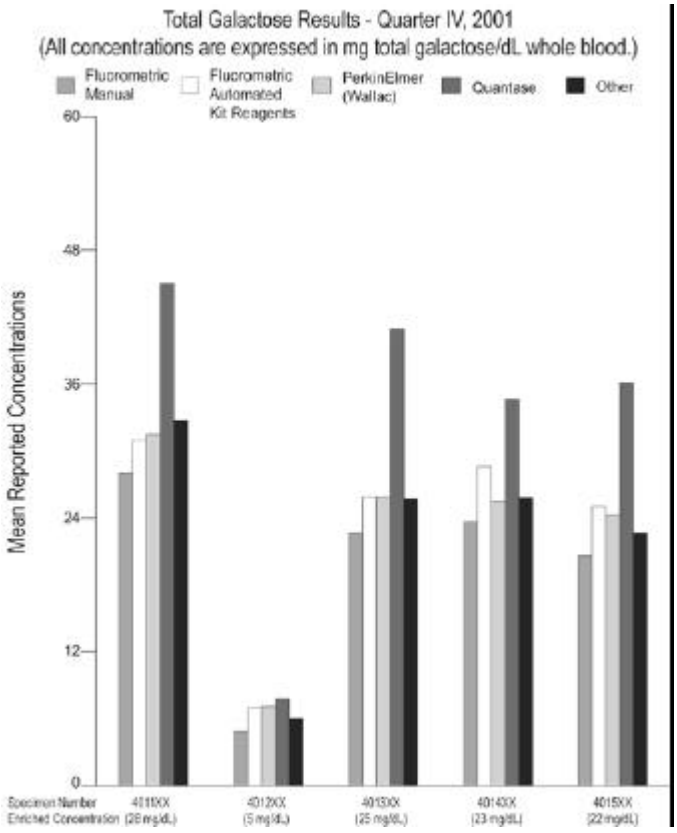
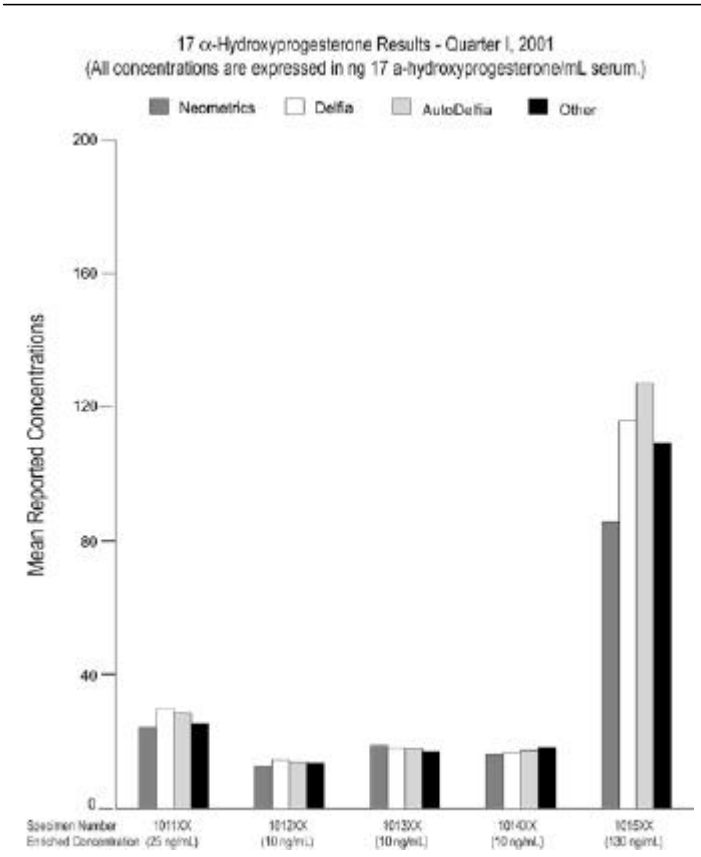
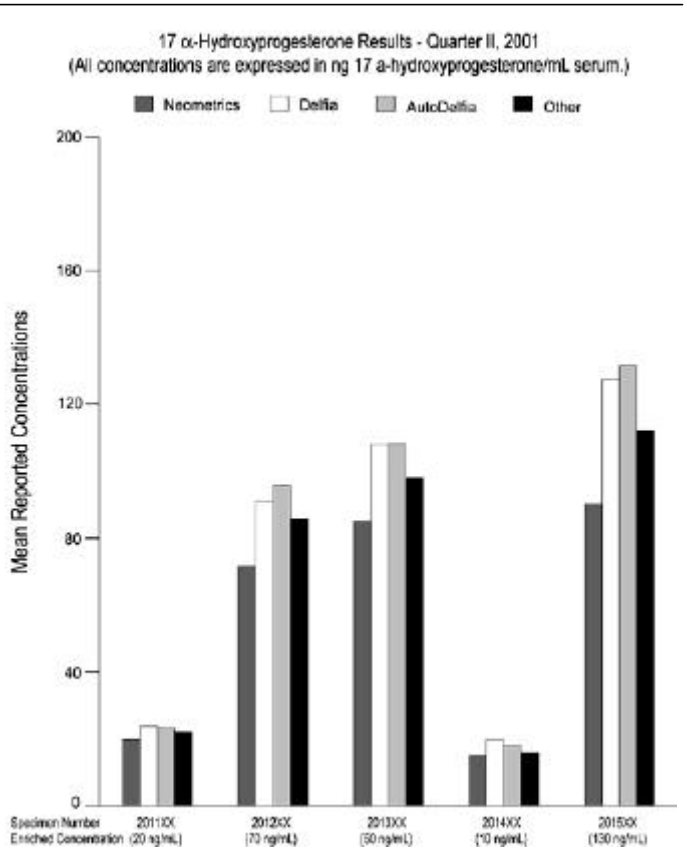
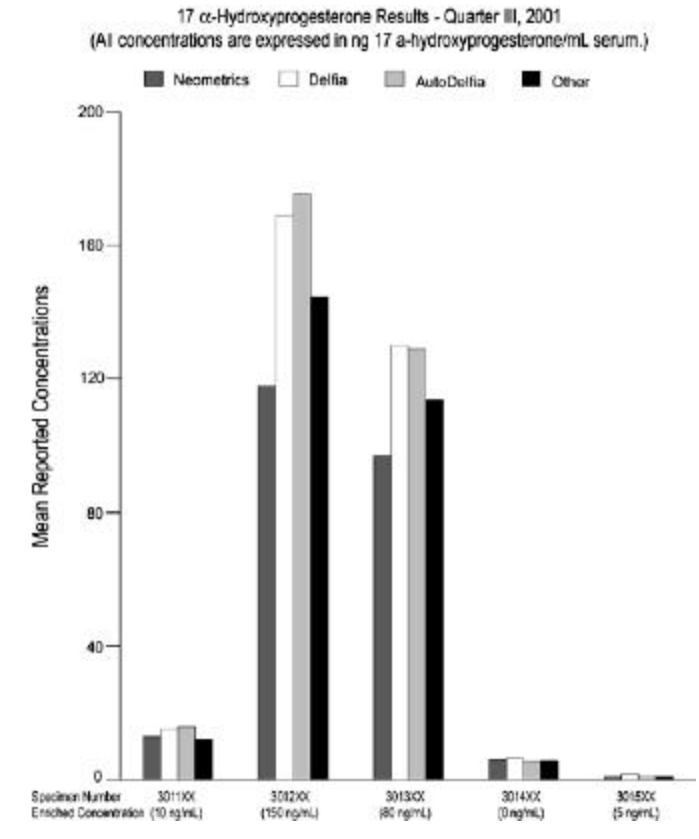


FIGURE 9e. 2001 Proficiency Testing Data
Mean Reported Concentrations of 17-OHP By Specimen Number







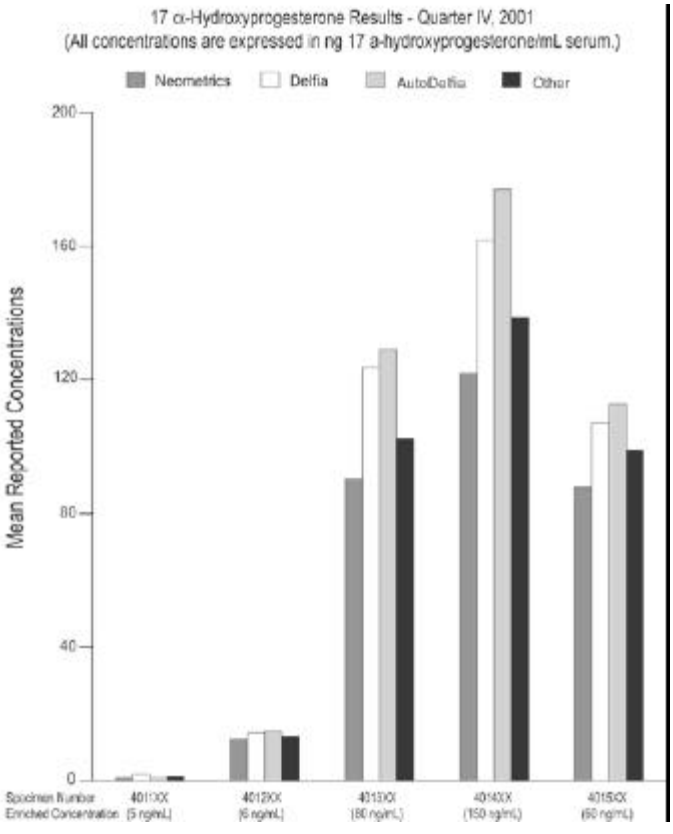


FIGURE 9f. 2001 Proficiency Testing Data
Mean Reported Concentrations of Leucine By Specimen Number

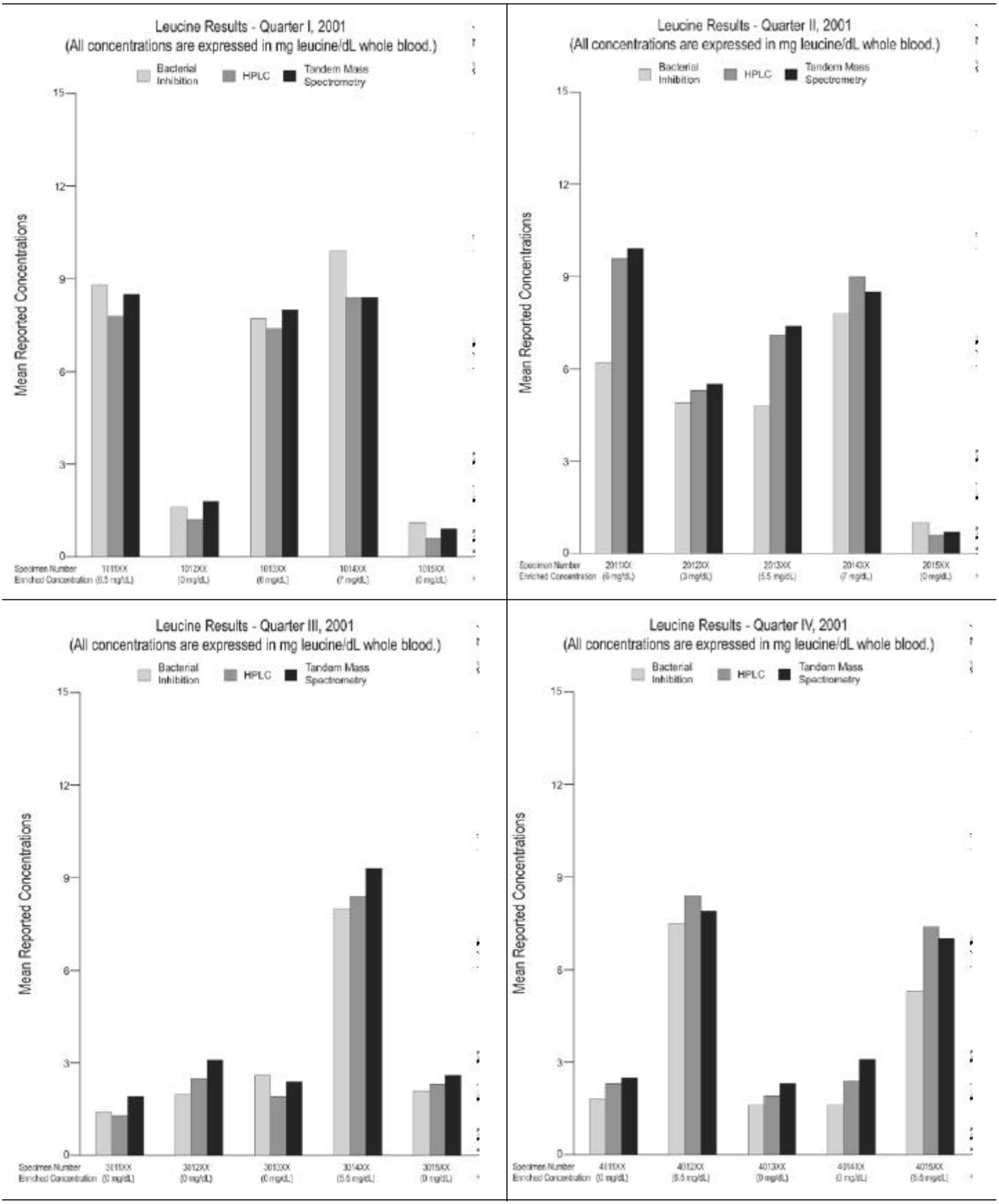
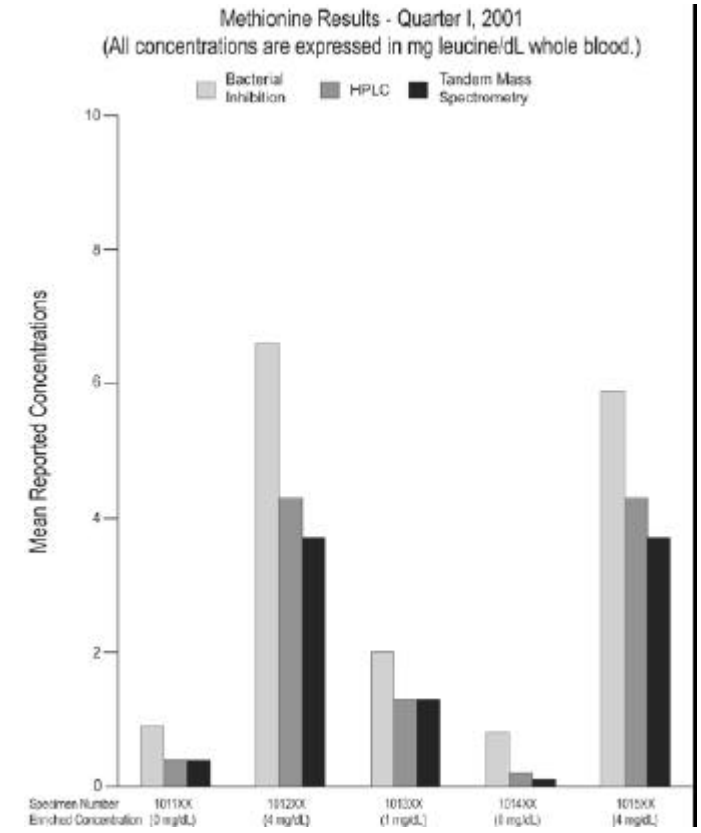
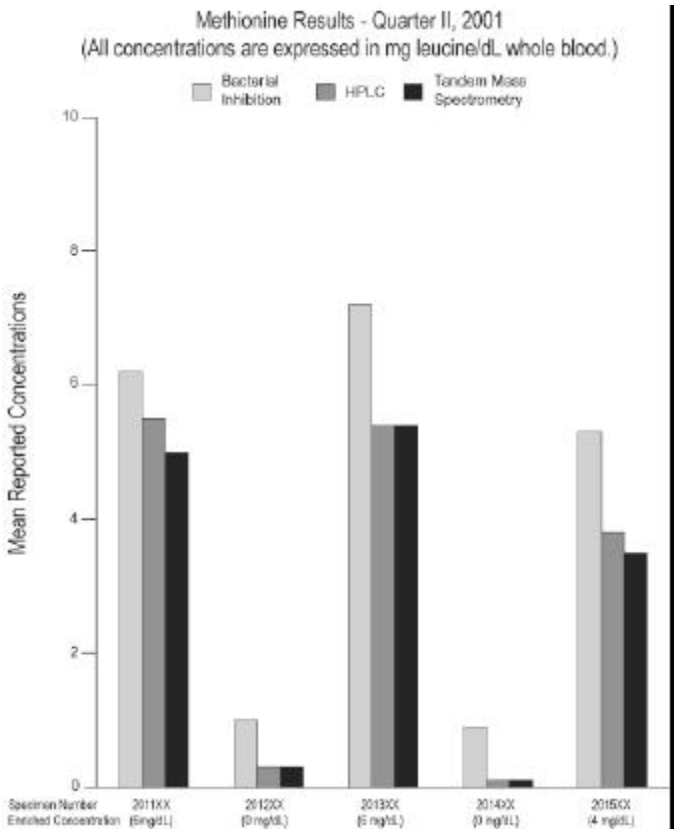
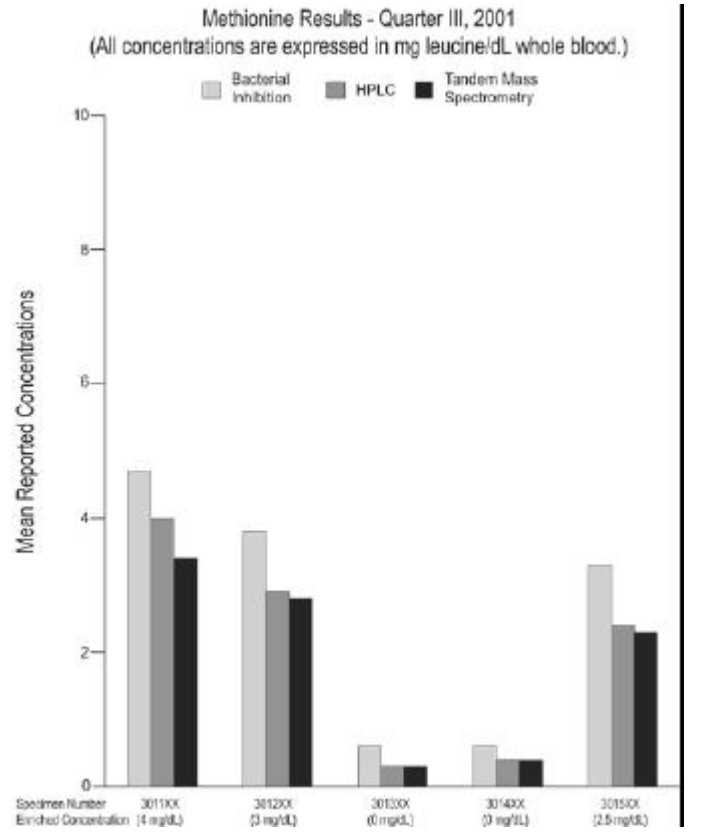


FIGURE 9g. 2001 Proficiency Testing Data
Mean Reported Concentrations of Methionine By Specimen Number







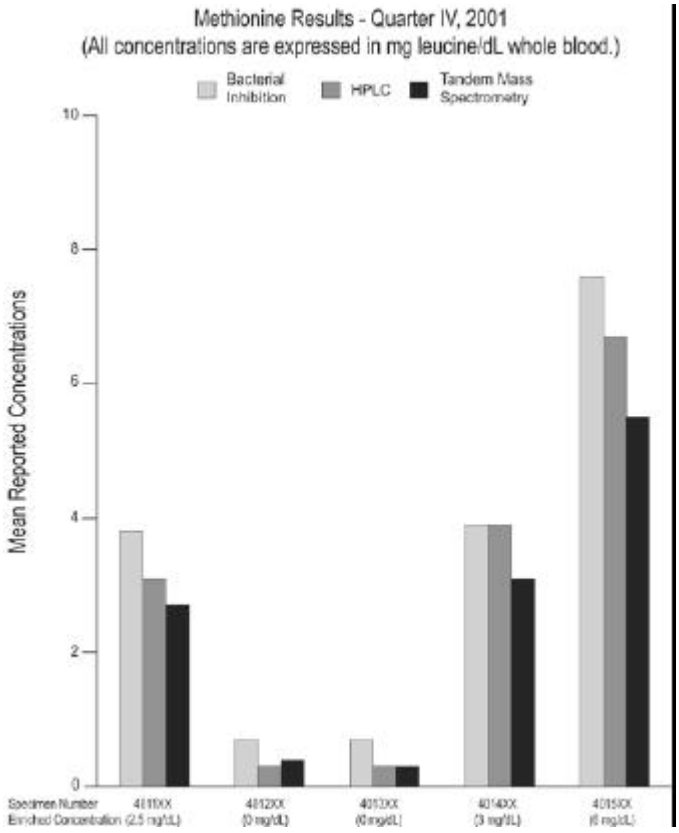


FIGURE 10. Reproducibility of Results Between Quarters for Different Methods

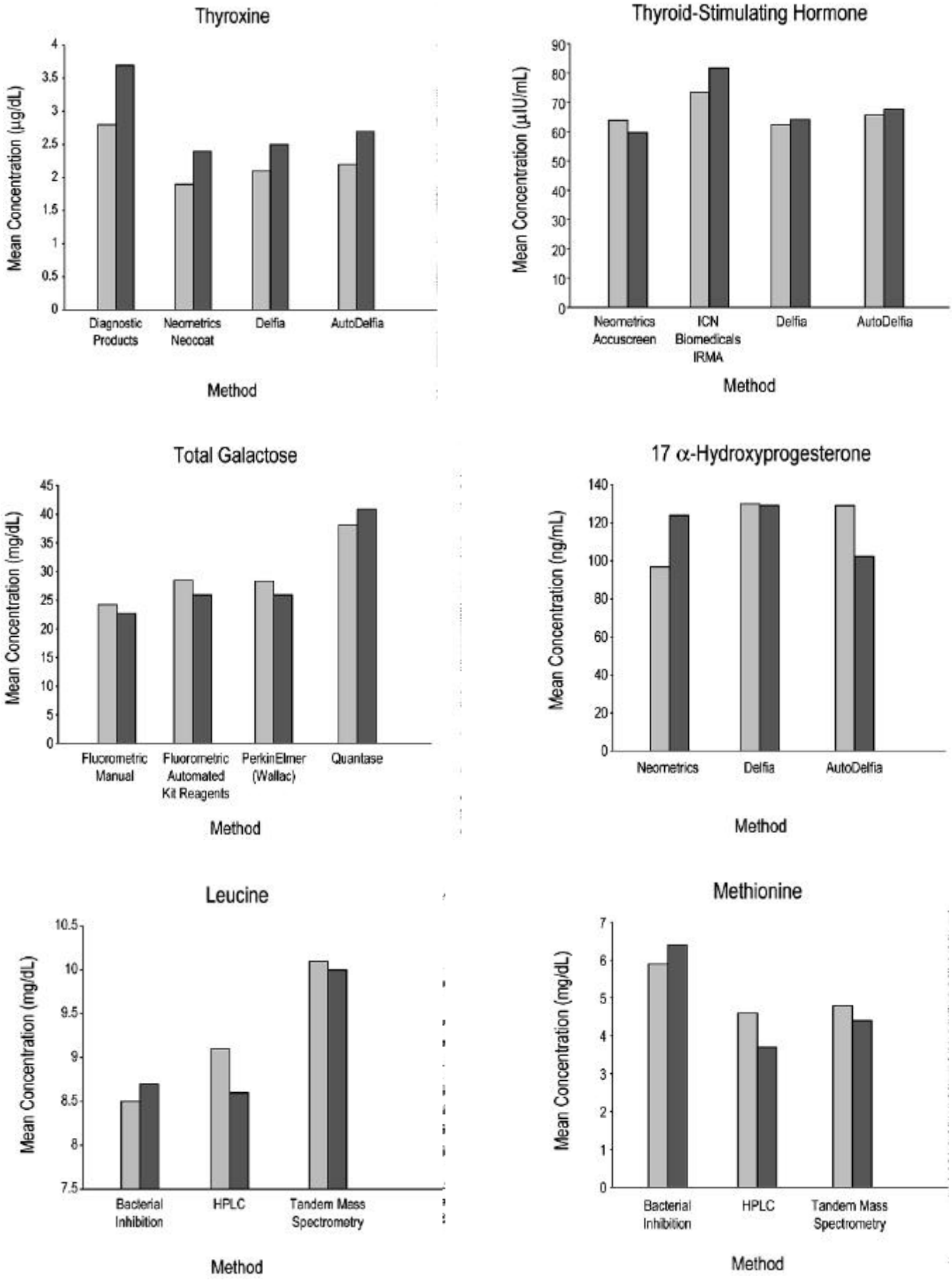


Figure 10 shows the reproducibility for the same specimen assayed during different quarters by selected methods. The time interval between reported values varies from one to four quarters but are the same interval for a single analyte. For most methods, the reproducibility is excellent. For Phe (not shown), essentially no difference was observed for the repeated specimen (6 mg/mL) for three of the four methods; however, one method was consistently higher.

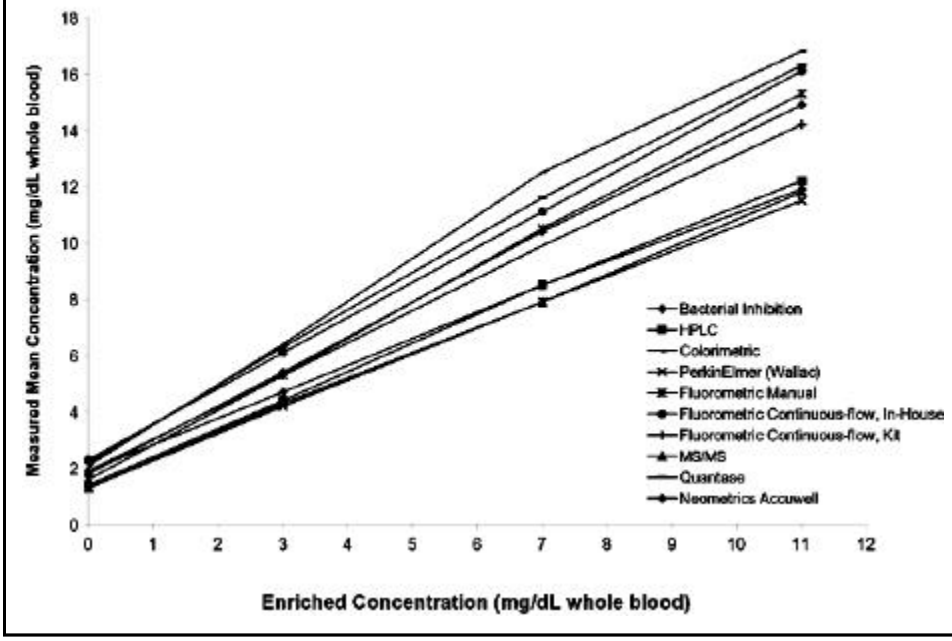
Table 1 shows the performance evaluation errors reported by disorder in 2001 for all qualitative assessments by domestic laboratories and by foreign laboratories. We applied the laboratory-reported specific cutoff values to our judgment algorithm for clinical assessments (see "Cutoffs" section). The rates for false-positive misclassifications were based on the number of distributed negative specimens, and the rates for false-negative misclassifications were based on the number of positive specimens. False-positive misclassifications, which are a cost-benefit issue and a credibility factor for follow-up programs, should be monitored and kept as low as possible. Many of the misclassifications were in the false-positive category, with false-positive rates ranging from 0% to 6.7%. For domestic laboratories, the rate was below 2% for seven of eight disorders; and for foreign laboratories, the rate was 2% or greater for seven of eight disorders. Screening programs are designed to avoid false-negative reports; this precautionary design, however, contributes to false-positive reports and may be the cause of many of the false-positive misclassifications. The false-negative rate, expected to be zero, ranged from 0% to 6.4%. False-negative classifications were reported for seven of the eight disorders, with the highest rate reported for biotinidase deficiency. For one disorder, no false-negative errors were reported for the domestic laboratories. A few of our PT specimens fell close to the decision level for classifications and thus rigorously tested the ability of laboratories to make the expected cutoff decision. Most specimens near the mean cutoff value are distributed as nongraded specimens and are not included in Table 1. Participants' data for these specimens are used to examine the relative analytical performance of the assays. Table 2 shows the performance errors for hemoglobinopathies. The percentage of errors for qualitative assessments for sickle cell disease and other hemoglobinopathies ranged from 0.5% to 4.7% for the error categories, with 50 of 58 laboratories correctly classifying all specimens. The classification errors are essentially the same for phenotype and clinical assessments within the domestic and foreign laboratory groups.

A few of our PT specimens fell close to the decision level for classification and thus rigorously tested the ability of laboratories to make the expected cutoff decision.

QUALITY CONTROL

For QC shipments of T_4, TSH, Phe, Gal, 17-OHP, Leu, and Met, each lot contained a different analyte concentration. To ensure that a laboratory received representative sheets of the production batch, we used a random number table to select the set of sheets from the production batch for each laboratory. The QC materials were distributed semiannually and included the blood-spot sheets, instructions for storage and analysis, and data-report forms. Data from five analytic runs of each lot and

FIGURE 11. Comparison of Different Methods for Detecting Phenylalanine in Dried-Blood Spots (Lots 041-044)



shipment were compiled in the midyear and annual summary reports that were distributed to each participant. Intervals between runs were not the same for all laboratories because each participant's reported data cover a different time span.

Figure 11 shows a performance comparison of different methods for measuring Phe from one set of QC materials distributed in 2001. The difference between the intercept value and zero represents the measured value for the endogenous level of Phe in all the specimens. Two different groupings occur among the methods with MS/MS, HPLC, bacterial-inhibition assay, and PerkinElmer (Wallac) in one group. The addition (see QC Tables 3a-3g for actual values) of the endogenous level (nonenriched specimens) to the enriched level yields the expected value. The reported QC data are summarized in Tables 3a-3g, which show the analyte by series of QC lots, the number of measurements (N), the mean values, and the standard deviations (SD) by kit or analytic method. In addition, we used a weighted linear regression analysis to examine the comparability by method of reported versus enriched concentrations. Linear regressions (Y-intercept and slope) were calculated by method for all analytic values within an analyte QC series. Values outside the 99% confidence limits (outliers) were excluded from the calculations.

Generally, slope values substantially different from 1.0 indicate that a method has an analytic bias.

Tables 3a-3g, which summarize reported QC results, provide data about method-related differences in analytic recoveries and method bias. Because we prepared each QC lot series from a single batch of hematocrit-adjusted, nonenriched blood, the endogenous concentration was the same for all specimens in a lot series. We calculated the within-laboratory SD component of the total SD and used the reported QC data from multiple analytic runs for regression analyses. We calculated the Y-intercept and slope in each table using all analyte concentrations within a lot series (e.g., lots 111, 112, and 113). Because only three or four concentrations of QC materials are available for each analyte, a bias error in any one pool can markedly influence the slope and intercept. The Y-intercept provides one measure of the endogenous concentration level for an analyte. One of the 17-OHP methods showed a higher-than-expected Y-intercept of 7.7 ng/mL. For Phe, Leu, and Met, participants also measured the endogenous concentrations by analyzing the nonenriched QC lots; the Y-intercepts and measured endogenous levels for Phe, Leu and Met were similar for most methods. Ideally, the slope should be 1.0, and most slopes were close to this value, ranging from 0.8 to 1.2; however, for one Phe method, two Gal methods, one Leu method, and one 17-OHP method, they were 1.4, 1.4, 0.7, and 0.7, respectively. These slope deviations may be related to analytic ranges for calibration curves or to low recoveries for one specimen in a three-or four-specimen QC set. Because the endogenous concentration was the same for all QC lots within a series, it should not affect the slope of the regression line among methods. Generally, slope values substantially different from 1.0 indicate that a method has an analytic bias.

REFERENCES

1. Hannon WH, Boyle J, Davin B, Marsden A, McCabe ERB, Schwartz M, et al. Blood collection on filter paper for neonatal screening programs. Third edition, approved standard. Wayne (PA): National Committee for Clinical Laboratory Standards; 1997 NCCLS Document LA4-A3.

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Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services or the Association of Public Health Laboratories.

TABLE 3a. 2001 Quality Control Data
Summaries of Statistical Analyses

17 a-HYDROXYPROGESTERONE (ng 17-OHP/mL serum)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 657 - Enriched 25 ng/mL serum						
Neometrics	108	25.5	1.8	2.1	7.7	0.7
Delfia	249	27.9	3.3	4.6	0.9	1.1
AutoDelfia	334	28.8	3.4	4.6	1.0	1.1
Other	116	24.4	3.0	4.8	4.7	0.9
Lot 658 - Enriched 50 ng/mL serum						
Neometrics	105	45.0	3.7	3.8	7.7	0.7
Delfia	244	56.8	6.4	7.3	0.9	1.1
AutoDelfia	333	57.4	6.5	8.8	1.0	1.1
Other	116	50.4	6.8	10.9	4.7	0.9
Lot 659 - Enriched 100 ng/mL serum						
Neometrics	105	80.5	10.7	12.4	7.7	0.7
Delfia	245	110.6	15.9	21.0	0.9	1.1
AutoDelfia	338	113.0	11.7	17.7	1.0	1.1
Other	118	89.9	14.4	22.3	4.7	0.9

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TABLE 3b. 2001 Quality Control Data
Summaries of Statistical Analyses

THYROXINE (µg T_4/dL serum)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 901 - Enriched 2 µg/dL serum						
Diagnostic Products	70	2.7	0.7	0.8	0.8	1.0
ICN Manual	79	2.5	0.4	0.6	0.7	0.9
Neometrics Accuscreen	60	3.3	0.6	0.7	1.3	1.0
Neometrics Neocoat	80	2.3	0.4	0.5	0.5	1.0
Neometrics Accuwell	68	3.1	0.6	0.8	1.2	1.0
Delfia	206	2.0	0.4	0.5	0.2	0.9
AutoDelfia	269	2.1	0.5	0.6	0.4	0.8
Other	59	2.4	0.7	0.7	0.4	1.0
Lot 902 - Enriched 5.5 µg/dL serum						
Diagnostic Products	69	6.4	1.2	1.3	0.8	1.0
ICN Manual	110	5.6	0.7	0.9	0.7	0.9
Neometrics Accuscreen	59	6.5	0.9	1.1	1.3	1.0
Neometrics Neocoat	79	6.0	0.7	0.7	0.5	1.0
Neometrics Accuwell	69	7.4	1.0	1.2	1.2	1.0
Delfia	205	4.9	0.8	1.0	0.2	0.9
AutoDelfia	271	5.1	0.8	1.0	0.4	0.8
Other	59	6.0	1.0	1.0	0.4	1.0
Lot 903 - Enriched 8 µg/dL serum						
Diagnostic Products	69	8.5	1.5	1.7	0.8	1.0
ICN Manual	109	7.9	0.7	0.9	0.7	0.9
Neometrics Accuscreen	59	9.1	1.3	1.4	1.3	1.0
Neometrics Neocoat	79	8.1	0.8	0.9	0.5	1.0
Neometrics Accuwell	69	9.3	1.8	2.0	1.2	1.0
Delfia	206	7.2	1.1	1.2	0.2	0.9
AutoDelfia	270	7.2	1.3	1.5	0.4	0.8
Other	60	8.4	1.2	1.2	0.4	1.0

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

THYROXINE (µg T_4/dL serum)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 001 - Enriched 2 µg/dL serum						
Diagnostic Products	28	2.5	0.6	0.7	0.4	1.1
ICN Manual	50	2.4	0.3	0.7	0.8	0.9
Neometrics Accuscreen	29	2.9	0.6	0.7	0.2	1.2
Neometrics Neocoat	30	2.7	0.6	0.9	1.1	0.9
Neometrics Accuwell	39	3.6	0.8	1.2	1.9	1.0
Delfia	97	2.1	0.5	0.5	0.4	0.8
AutoDelfia	174	2.3	0.4	0.6	0.7	0.9
Other	20	2.0	0.4	0.4	0.0	1.0
Lot 002 - Enriched 5.5 µg/dL serum						
Diagnostic Products	30	6.7	1.7	1.7	0.4	1.1
ICN Manual	59	6.0	0.7	0.7	0.8	0.9
Neometrics Accuscreen	29	6.0	0.7	0.8	0.2	1.2
Neometrics Neocoat	30	6.7	0.4	1.0	1.1	0.9
Neometrics Accuwell	40	8.0	1.1	1.8	1.9	1.0
Delfia	97	5.0	0.6	0.6	0.4	0.8
AutoDelfia	173	5.5	0.8	1.2	0.7	0.9
Other	19	5.9	1.2	1.2	0.0	1.0
Lot 003 - Enriched 8 µg/dL serum						
Diagnostic Products	30	9.1	1.7	1.9	0.4	1.1
ICN Manual	59	7.8	0.8	1.0	0.8	0.9
Neometrics Accuscreen	29	10.2	2.3	2.3	0.2	1.2
Neometrics Neocoat	30	8.2	0.6	0.8	1.1	0.9
Neometrics Accuwell	40	9.4	1.1	1.5	1.9	1.0
Delfia	98	7.1	0.8	1.0	0.4	0.8
AutoDelfia	170	7.4	0.7	1.9	0.7	0.9
Other	20	8.3	0.9	1.1	0.0	1.0

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TABLE 3c. 2001 Quality Control Data
Summaries of Statistical Analyses

THYROID-STIMULATING HORMONE (µIU TSH/mL serum)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 011 - Enriched 25 µIU/mL serum						
Diagnostic Products	89	28.0	3.0	3.7	-0.6	1.1
Neometrics Accuscreen	89	24.8	3.9	3.9	2.9	0.9
Neometrics Accuwell	60	23.4	2.3	2.7	0.7	0.9
ICN Biomedicals IRMA	178	29.6	4.0	7.7	2.0	1.1
Delfia	819	24.0	4.1	6.5	-0.3	1.0
AutoDelfia	512	24.9	2.9	4.0	-1.2	1.0
Thermo Labsystems	50	26.1	4.3	6.5	2.0	1.1
In House	95	28.4	5.2	11.4	-1.0	1.2
Other	526	25.4	4.2	7.7	-1.3	1.1
Lot 012 - Enriched 40 µIU/mL serum						
Diagnostic Products	88	44.2	4.6	5.0	-0.6	1.1
Neometrics Accuscreen	89	37.1	4.4	5.2	2.9	0.9
Neometrics Accuwell	59	36.2	4.2	4.5	0.7	0.9
ICN Biomedicals IRMA	177	46.0	6.6	12.3	2.0	1.1
Delfia	816	38.1	6.1	9.4	-0.3	1.0
AutoDelfia	512	39.7	4.4	6.1	-1.2	1.0
Thermo Labsystems	49	47.1	6.0	6.1	2.0	1.1
In House	97	46.8	6.6	16.8	-1.0	1.2
Other	540	41.8	5.7	11.4	-1.3	1.1
Diagnostic Products	88	89.9	7.4	8.5	-0.6	1.1
Neometrics Accuscreen	89	72.1	8.2	9.9	2.9	0.9
Neometrics Accuwell	60	72.6	7.0	8.3	0.7	0.9
ICN Biomedicals IRMA	176	90.2	14.0	19.3	2.0	1.1
Delfia	819	77.1	9.2	16.2	-0.3	1.0
AutoDelfia	516	81.3	8.3	11.7	-1.2	1.0
Thermo Labsystems	50	85.3	9.3	10.7	2.0	1.1
In House	98	93.9	14.8	36.6	-1.0	1.2
Other	543	84.6	11.0	21.3	-1.3	1.1

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

THYROID-STIMULATING HORMONE (µIU TSH/mL serum)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 111 - Enriched 25 µIU/mL serum						
Diagnostic Products	59	29.0	2.6	3.0	0.0	1.2
Neometrics Accuscreen	40	22.7	4.2	4.6	-1.5	1.0
Neometrics Accuwell	28	21.5	3.4	3.8	-0.7	0.9
ICN Biomedicals IRMA	87	29.3	4.1	8.4	4.9	1.0
Delfia	387	24.5	4.3	6.1	0.2	1.0
AutoDelfia	306	23.9	2.3	3.6	0.9	0.9
Thermo Labsystems	20	24.2	3.0	8.9	-1.2	1.1
In House	40	28.3	4.1	6.2	0.6	1.1
Other	280	24.1	4.4	7.3	-0.8	1.0
Lot 112 - Enriched 40 µIU/mL serum						
Diagnostic Products	57	46.3	4.0	6.1	0.0	1.2
Neometrics Accuscreen	40	37.3	5.4	5.7	-1.5	1.0
Neometrics Accuwell	30	36.5	3.9	4.4	-0.7	0.9
ICN Biomedicals IRMA	86	44.7	5.7	12.2	4.9	1.0
Delfia	385	39.2	5.1	8.4	0.2	1.0
AutoDelfia	303	38.4	3.9	5.5	0.9	0.9
Thermo Labsystems	20	44.5	4.7	9.5	-1.2	1.1
In House	40	46.2	5.0	10.9	0.6	1.1
Other	281	38.9	5.3	10.1	-0.8	1.0
Lot 113 - Enriched 80 µIU/mL serum						
Diagnostic Products	59	92.7	10.6	13.5	0.0	1.2
Neometrics Accuscreen	39	76.0	8.2	9.1	-1.5	1.0
Neometrics Accuwell	30	72.0	7.6	7.8	-0.7	0.9
ICN Biomedicals IRMA	86	83.7	9.3	18.6	4.9	1.0
Delfia	384	78.2	9.4	14.8	0.2	1.0
AutoDelfia	302	75.1	7.1	10.0	0.9	0.9
Thermo Labsystems	20	84.8	5.5	9.0	-1.2	1.1
In House	40	90.5	9.2	21.2	0.6	1.1
Other	281	78.6	10.7	19.4	-0.8	1.0

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TABLE 3d. 2001 Quality Control Data
Summaries of Statistical Analyses

PHENYLALANINE (mg Phe/dL whole blood)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 041 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition	152	1.9	0.4	0.6	2.0	0.9
HPLC	78	1.4	0.2	0.2	1.5	1.0
Colorimetric	108	2.1	0.3	0.6	2.3	1.4
PerkinElmer (Wallac)	234	1.3	0.2	0.3	1.4	0.9
Fluorometric Manual	59	1.6	0.5	0.7	1.6	1.3
Fluor Cont Flo, In-house	20	2.3	0.2	0.2	2.3	1.3
Fluor Cont Flo, Kit	129	1.9	0.2	0.5	1.9	1.1
Tandem Mass Spec	49	1.3	0.1	0.2	1.3	0.9
Quantase	118	2.2	0.4	0.7	2.4	1.3
Neometrics Accuwell	50	1.8	0.3	0.4	1.8	1.2
Other	60	1.6	0.5	0.8	1.6	1.0
Lot 042 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition	170	4.7	0.7	1.1	2.0	0.9
HPLC	88	4.4	0.4	0.6	1.5	1.0
Colorimetric	108	6.4	0.5	1.1	2.3	1.4
PerkinElmer (Wallac)	237	4.2	0.4	0.6	1.4	0.9
Fluorometric Manual	60	5.3	1.4	1.9	1.6	1.3
Fluor Cont Flo, In-house	20	6.1	0.7	0.7	2.3	1.3
Fluor Cont Flo, Kit	129	5.3	0.3	0.7	1.9	1.1
Tandem Mass Spec	50	4.3	0.3	0.5	1.3	0.9
Quantase	117	6.3	0.6	1.3	2.4	1.3
Neometrics Accuwell	50	5.4	0.4	0.6	1.8	1.2
Other	59	4.6	0.6	0.8	1.6	1.0
Lot 043 - Enriched 7 mg/dL whole blood						
Bacterial Inhibition	168	8.5	1.4	1.8	2.0	0.9
HPLC	79	8.5	0.7	1.1	1.5	1.0
Colorimetric	107	12.5	1.0	2.0	2.3	1.4
PerkinElmer (Wallac)	235	7.9	0.8	1.2	1.4	0.9
Fluorometric Manual	48	10.5	1.0	2.1	1.6	1.3
Fluor Cont Flo, In-house	20	11.1	1.0	1.0	2.3	1.3
Fluor Cont Flo, Kit	129	9.9	0.7	1.1	1.9	1.1
Tandem Mass Spec	50	7.9	0.8	1.0	1.3	0.9
Quantase	120	11.6	0.9	2.1	2.4	1.3
Neometrics Accuwell	50	10.4	0.7	0.9	1.8	1.2
Other	59	8.8	0.9	1.6	1.6	1.0

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

PHENYLALANINE (mg Phe/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 044 - Enriched 11 mg/dL whole blood						
Bacterial Inhibition	159	11.9	1.3	2.3	2.0	0.9
HPLC	88	12.2	1.0	1.8	1.5	1.0
Colorimetric	108	16.8	1.1	3.3	2.3	1.4
PerkinElmer (Wallac)	236	11.5	1.1	1.7	1.4	0.9
Fluorometric Manual	49	15.3	1.6	3.7	1.6	1.3
Fluor Cont Flo, In-house	20	16.1	0.8	1.8	2.3	1.3
Fluor Cont Flo, Kit	127	14.2	1.3	2.1	1.9	1.1
Tandem Mass Spec	50	11.8	1.2	1.6	1.3	0.9
Quantase	120	16.3	1.3	3.0	2.4	1.3
Neometrics Accuwell	49	14.9	1.1	1.3	1.8	1.2
Other	59	12.7	1.2	2.1	1.6	1.0
Lot 121 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition	343	1.8	0.4	0.6	1.8	0.9
HPLC	118	1.5	0.2	0.2	1.6	0.9
Colorimetric	199	2.1	0.3	0.5	2.2	1.3
PerkinElmer (Wallac)	471	1.5	0.3	0.4	1.5	0.9
Fluorometric Manual	128	1.8	0.5	0.7	1.7	1.1
Fluor Cont Flo, In-house	58	2.1	0.2	0.3	2.1	1.2
Fluor Cont Flo, Kit	238	1.9	0.2	0.5	1.9	1.1
Tandem Mass Spec	157	1.5	0.2	0.4	1.6	1.0
Quantase	236	2.0	0.4	0.8	2.1	1.2
Neometrics Accuwell	98	2.0	0.2	0.4	2.0	1.2
Other	115	1.9	0.4	0.7	2.0	1.0
Lot 122 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition	376	4.4	0.8	1.1	1.8	0.9
HPLC	139	4.4	0.6	0.9	1.6	0.9
Colorimetric	190	6.2	0.6	2.0	2.2	1.3
PerkinElmer (Wallac)	473	4.2	0.5	0.6	1.5	0.9
Fluorometric Manual	129	5.0	0.8	1.4	1.7	1.1
Fluor Cont Flo, In-house	59	5.7	0.5	0.7	2.1	1.2
Fluor Cont Flo, Kit	238	5.2	0.4	0.8	1.9	1.1
Tandem Mass Spec	155	4.5	0.5	1.1	1.6	1.0
Quantase	239	5.7	0.8	1.4	2.1	1.2
Neometrics Accuwell	97	5.7	0.5	0.8	2.0	1.2
Other	129	4.9	0.5	1.0	2.0	1.0

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

PHENYLALANINE (mg Phe/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 123 - Enriched 7 mg/dL whole blood						
Bacterial Inhibition	372	8.3	1.2	1.7	1.8	0.9
HPLC	115	8.2	0.8	1.1	1.6	0.9
Colorimetric	193	11.9	1.3	3.0	2.2	1.3
PerkinElmer (Wallac)	473	7.9	0.8	1.1	1.5	0.9
Fluorometric Manual	129	9.7	1.2	2.0	1.7	1.1
Fluor Cont Flo, In-house	60	10.6	0.8	1.4	2.1	1.2
Fluor Cont Flo, Kit	239	9.5	0.8	1.4	1.9	1.1
Tandem Mass Spec	160	8.4	1.1	2.1	1.6	1.0
Quantase	240	10.8	1.3	2.4	2.1	1.2
Neometrics Accuwell	95	10.1	1.2	1.7	2.0	1.2
Other	128	9.2	1.0	1.6	2.0	1.0
Lot 124 - Enriched 11 mg/dL whole blood						
Bacterial Inhibition	370	11.6	1.5	2.0	1.8	0.9
HPLC	137	11.7	1.2	1.7	1.6	0.9
Colorimetric	199	16.5	1.7	2.9	2.2	1.3
PerkinElmer (Wallac)	471	11.4	1.2	1.5	1.5	0.9
Fluorometric Manual	130	14.2	2.0	3.4	1.7	1.1
Fluor Cont Flo, In-house	58	15.3	1.3	2.0	2.1	1.2
Fluor Cont Flo, Kit	235	13.8	1.0	2.0	1.9	1.1
Tandem Mass Spec	151	12.0	1.3	2.9	1.6	1.0
Quantase	238	15.2	1.7	3.1	2.1	1.2
Neometrics Accuwell	89	14.9	1.1	1.2	2.0	1.2
Other	126	12.9	1.1	1.7	2.0	1.0
Lot 141 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition	177	1.7	0.4	0.8	1.8	1.0
HPLC	59	1.3	0.1	0.2	1.4	1.0
Colorimetric	110	2.2	0.4	0.6	2.4	1.3
PerkinElmer (Wallac)	234	1.2	0.3	0.3	1.3	1.0
Fluorometric Manual	58	1.6	0.5	1.7	1.7	1.2
Fluor Cont Flo, In-house	30	2.0	0.2	0.4	1.9	1.3
Fluor Cont Flo, Kit	120	1.8	0.2	0.5	1.8	1.2
Tandem Mass Spec	119	1.4	0.2	0.3	1.4	1.1
Quantase	137	1.9	0.5	0.8	1.9	1.3
Neometrics Accuwell	49	2.1	0.3	0.3	2.2	1.3
Other	40	1.7	0.3	0.4	1.8	1.2

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

PHENYLALANINE (mg Phe/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 142 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition	207	4.6	0.7	1.1	1.8	1.0
HPLC	69	4.3	0.4	0.5	1.4	1.0
Colorimetric	101	6.5	0.9	2.2	2.4	1.3
PerkinElmer (Wallac)	238	4.3	0.5	0.6	1.3	1.0
Fluorometric Manual	58	5.1	0.6	3.1	1.7	1.2
Fluor Cont Flo, In-house	30	5.7	0.4	0.8	1.9	1.3
Fluor Cont Flo, Kit	120	5.4	0.5	0.9	1.8	1.2
Tandem Mass Spec	114	4.6	0.5	1.2	1.4	1.1
Quantase	139	5.9	0.8	1.5	1.9	1.3
Neometrics Accuwell	48	6.1	0.5	0.8	2.2	1.3
Other	50	5.4	0.4	1.2	1.8	1.2
Lot 143 - Enriched 7 mg/dL whole blood						
Bacterial Inhibition	206	8.9	1.0	1.5	1.8	1.0
HPLC	59	8.7	0.6	1.0	1.4	1.0
Colorimetric	111	11.9	1.4	2.5	2.4	1.3
PerkinElmer (Wallac)	234	8.3	1.0	1.2	1.3	1.0
Fluorometric Manual	60	10.1	1.0	3.0	1.7	1.2
Fluor Cont Flo, In-house	29	10.8	0.8	1.3	1.9	1.3
Fluor Cont Flo, Kit	120	10.3	0.9	1.7	1.8	1.2
Tandem Mass Spec	117	8.9	0.9	2.5	1.4	1.1
Quantase	139	11.4	1.6	2.7	1.9	1.3
Neometrics Accuwell	47	11.8	1.3	2.0	2.2	1.3
Other	49	10.1	0.8	1.8	1.8	1.2
Lot 144 - Enriched 11 mg/dL whole blood						
Bacterial Inhibition	203	12.1	1.4	2.2	1.8	1.0
HPLC	70	12.0	1.1	1.5	1.4	1.0
Colorimetric	108	16.7	2.1	3.6	2.4	1.3
PerkinElmer (Wallac)	234	12.2	1.3	1.6	1.3	1.0
Fluorometric Manual	58	14.1	1.8	8.2	1.7	1.2
Fluor Cont Flo, In-house	30	16.2	1.1	1.9	1.9	1.3
Fluor Cont Flo, Kit	120	15.0	1.2	2.3	1.8	1.2
Tandem Mass Spec	118	13.1	1.3	3.2	1.4	1.1
Quantase	140	16.5	2.0	3.9	1.9	1.3
Neometrics Accuwell	40	16.2	0.9	1.2	2.2	1.3
Other	50	14.5	1.0	2.5	1.8	1.2

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TABLE 3e. 2001 Quality Control Data
Summaries of Statistical Analyses

TOTAL GALACTOSE (mg Gal/dL whole blood)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 041 - Enriched 5 mg/dL whole blood						
Colorimetric	30	8.1	0.5	1.2	0.6	1.4
PerkinElmer (Wallac)	87	6.5	1.0	1.3	1.8	1.0
Fluorometric Manual	147	5.4	0.8	2.1	-0.2	1.0
Fluor Cont Flo, Kit	59	6.9	0.8	0.8	1.6	1.1
Neometrics Accuwell	40	7.8	0.6	1.1	0.9	1.3
Quantase	50	6.2	0.9	1.2	-0.6	1.3
Other	20	5.5	2.1	2.1	-1.6	1.3
Lot 042 - Enriched 10 mg/dL whole blood						
Colorimetric	30	15.0	0.7	3.4	0.6	1.4
PerkinElmer (Wallac)	89	12.0	1.4	2.2	1.8	1.0
Fluorometric Manual	157	10.0	1.1	2.9	-0.2	1.0
Fluor Cont Flo, Kit	59	12.2	0.9	1.0	1.6	1.1
Neometrics Accuwell	39	13.5	1.1	1.7	0.9	1.3
Quantase	50	12.5	1.5	2.4	-0.6	1.3
Other	20	11.0	3.4	4.1	-1.6	1.3
Lot 043 - Enriched 15 mg/dL whole blood						
Colorimetric	30	22.0	1.2	6.7	0.6	1.4
PerkinElmer (Wallac)	88	17.1	2.2	2.6	1.8	1.0
Fluorometric Manual	157	14.7	1.4	3.6	-0.2	1.0
Fluor Cont Flo, Kit	60	17.3	1.0	1.0	1.6	1.1
Neometrics Accuwell	39	19.8	1.7	3.0	0.9	1.3
Quantase	50	20.3	2.1	4.0	-0.6	1.3
Other	20	19.2	3.5	5.1	-1.6	1.3

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TOTAL GALACTOSE (mg Gal/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 044 - Enriched 30 mg/dL whole blood						
Colorimetric	30	44.2	6.1	9.6	0.6	1.4
PerkinElmer (Wallac)	90	31.7	2.9	3.4	1.8	1.0
Fluorometric Manual	158	30.9	3.3	5.4	-0.2	1.0
Fluor Cont Flo, Kit	58	33.2	2.1	2.6	1.6	1.1
Neometrics Accuwell	40	39.6	4.0	6.2	0.9	1.3
Quantase	50	39.7	5.6	10.6	-0.6	1.3
Other	20	38.6	8.7	12.7	-1.6	1.3
Lot 121 - Enriched 5 mg/dL whole blood						
Colorimetric	70	6.8	1.1	1.5	0.5	1.3
PerkinElmer (Wallac)	187	6.9	1.3	1.7	2.4	1.0
Fluorometric Manual	257	5.2	0.7	2.0	0.0	1.0
Fluor Cont Flo, Kit	116	6.9	0.6	0.7	1.7	1.1
Neometrics Accuwell	70	7.6	0.7	0.8	1.1	1.3
Quantase	90	5.8	0.9	1.3	-0.1	1.3
Other	50	5.6	1.5	1.8	-0.7	1.2
Lot 122 - Enriched 10 mg/dL whole blood						
Colorimetric	70	13.2	1.8	3.2	0.5	1.3
PerkinElmer (Wallac)	188	12.8	1.7	2.1	2.4	1.0
Fluorometric Manual	276	10.3	1.0	2.8	0.0	1.0
Fluor Cont Flo, Kit	118	12.5	0.8	1.2	1.7	1.1
Neometrics Accuwell	70	14.2	1.0	1.3	1.1	1.3
Quantase	90	12.7	1.7	2.6	-0.1	1.3
Other	50	11.4	2.5	3.1	-0.7	1.2

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TOTAL GALACTOSE (mg Gal/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 123 - Enriched 15 mg/dL whole blood						
Colorimetric	70	19.6	1.9	5.8	0.5	1.3
PerkinElmer (Wallac)	190	18.2	2.0	2.6	2.4	1.0
Fluorometric Manual	272	15.5	1.5	3.8	0.0	1.0
Fluor Cont Flo, Kit	119	17.8	1.2	1.5	1.7	1.1
Neometrics Accuwell	69	20.2	1.6	2.0	1.1	1.3
Quantase	89	19.2	2.7	4.1	-0.1	1.3
Other	48	16.4	2.2	3.9	-0.7	1.2
Lot 124 - Enriched 30 mg/dL whole blood						
Colorimetric	70	38.3	3.5	7.9	0.5	1.3
PerkinElmer (Wallac)	185	32.4	3.6	4.1	2.4	1.0
Fluorometric Manual	271	31.0	3.1	4.3	0.0	1.0
Fluor Cont Flo, Kit	118	33.7	2.7	3.4	1.7	1.1
Neometrics Accuwell	70	40.0	3.0	4.1	1.1	1.3
Quantase	90	37.5	3.4	7.9	-0.1	1.3
Other	50	35.5	6.7	9.1	-0.7	1.2
Lot 141 - Enriched 5 mg/dL whole blood						
Colorimetric	70	7.8	0.9	2.5	1.6	1.3
PerkinElmer (Wallac)	89	6.4	1.3	1.5	1.4	1.0
Fluorometric Manual	106	5.5	0.7	2.1	0.1	1.1
Fluor Cont Flo, Kit	58	8.0	0.7	0.9	2.2	1.1
Neometrics Accuwell	30	8.1	0.6	0.7	0.9	1.4
Quantase	39	7.5	1.3	2.8	0.4	1.4
Other	20	6.5	0.6	3.0	1.6	1.1

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TOTAL GALACTOSE (mg Gal/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 142 - Enriched 10 mg/dL whole blood						
Colorimetric	70	15.0	1.3	2.9	1.6	1.3
PerkinElmer (Wallac)	90	11.5	1.5	1.7	1.4	1.0
Fluorometric Manual	116	10.9	0.8	2.6	0.1	1.1
Fluor Cont Flo, Kit	58	13.5	1.1	1.6	2.2	1.1
Neometrics Accuwell	29	14.6	0.9	1.1	0.9	1.4
Quantase	39	13.9	1.7	3.7	0.4	1.4
Other	20	13.2	1.0	3.2	1.6	1.1
Lot 143 - Enriched 15 md/dL whole blood						
Colorimetric	70	20.8	1.7	4.0	1.6	1.3
PerkinElmer (Wallac)	87	18.1	2.2	2.8	1.4	1.0
Fluorometric Manual	114	16.2	1.0	3.2	0.1	1.1
Fluor Cont Flo, Kit	59	19.3	1.7	2.2	2.2	1.1
Neometrics Accuwell	30	20.9	1.6	2.1	0.9	1.4
Quantase	40	21.3	2.1	5.6	0.4	1.4
Other	20	18.8	1.2	5.4	1.6	1.1
Lot 144 - Enriched 30 mg/dL whole blood						
Colorimetric	69	40.4	4.3	7.4	1.6	1.3
PerkinElmer (Wallac)	87	32.4	2.8	3.7	1.4	1.0
Fluorometric Manual	105	32.6	2.8	5.6	0.1	1.1
Fluor Cont Flo, Kit	59	36.4	2.4	3.4	2.2	1.1
Neometrics Accuwell	30	42.3	2.7	3.4	0.9	1.4
Quantase	30	42.0	4.4	11.4	0.4	1.4
Other	20	34.5	3.5	3.6	1.6	1.1

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TABLE 3f. 2001 Quality Control Data
Summaries of Statistical Analyses

LEUCINE (mg Leu/dL whole blood)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 041 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition Assays	69	2.4	0.5	0.8	2.3	0.7
HPLC	27	2.1	0.3	0.5	2.0	1.0
Tandem Mass Spec	40	2.6	0.3	0.4	2.7	0.9
Other	30	2.2	0.4	0.7	2.4	0.8
Lot 042 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition Assays	68	4.5	0.6	0.9	2.3	0.7
HPLC	27	5.0	0.4	0.7	2.0	1.0
Tandem Mass Spec	40	5.5	0.5	0.7	2.7	0.9
Other	30	4.9	0.4	0.4	2.4	0.8
Lot 043 - Enriched 7 mg/dL whole blood						
Bacterial Inhibition Assays	69	6.8	0.7	1.2	2.3	0.7
HPLC	28	9.2	0.6	1.5	2.0	1.0
Tandem Mass Spec	40	9.2	0.8	1.2	2.7	0.9
Other	30	8.7	0.8	1.1	2.4	0.8
Lot 044 - Enriched 11 mg/dL whole blood						
Bacterial Inhibition Assays	70	10.2	1.1	2.8	2.3	0.7
HPLC	28	13.5	0.9	2.2	2.0	1.0
Tandem Mass Spec	39	12.6	1.0	1.4	2.7	0.9
Other	30	11.4	0.6	1.2	2.4	0.8

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

LEUCINE (mg Leu/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 121 Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition Assays	140	1.9	0.5	0.8	2.1	0.8
HPLC	78	2.1	0.2	0.3	2.1	0.9
Tandem Mass Spec	116	2.5	0.3	0.6	2.6	0.9
Other	69	2.5	0.5	0.7	2.7	0.8
Lot 122 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition Assays	146	4.7	0.9	1.1	2.1	0.8
HPLC	79	5.0	0.5	0.7	2.1	0.9
Tandem Mass Spec	117	5.2	0.6	1.3	2.6	0.9
Other	68	5.3	0.7	0.8	2.7	0.8
Lot 123 - Enriched 7 mg/dL whole blood						
Bacterial Inhibition Assays	146	7.2	1.0	1.4	2.1	0.8
HPLC	77	8.6	0.6	1.2	2.1	0.9
Tandem Mass Spec	116	8.9	0.9	2.3	2.6	0.9
Other	69	8.6	1.0	1.4	2.7	0.8
Lot 124 - Enriched 11 mg/dL whole blood						
Bacterial Inhibition Assays	138	10.5	1.7	2.7	2.1	0.8
HPLC	79	12.6	0.9	1.7	2.1	0.9
Tandem Mass Spec	117	12.2	1.3	3.1	2.6	0.9
Other	69	11.5	1.4	2.2	2.7	0.8

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

LEUCINE (mg Leu/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 141 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition Assays	76	1.8	0.7	1.3	2.0	0.9
HPLC	50	1.7	0.2	0.2	1.7	1.1
Tandem Mass Spec	89	2.5	0.3	0.6	2.5	1.0
Other	40	2.3	0.6	0.8	2.3	0.8
Lot 142 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition Assays	88	4.8	0.8	1.4	2.0	0.9
HPLC	50	4.7	0.3	0.6	1.7	1.1
Tandem Mass Spec	88	5.4	0.6	1.3	2.5	1.0
Other	30	4.7	0.6	1.0	2.3	0.8
Lot 143 - Enriched 7 mg/dL whole blood						
Bacterial Inhibition Assays	83	8.2	1.1	2.4	2.0	0.9
HPLC	49	9.3	0.6	1.0	1.7	1.1
Tandem Mass Spec	88	9.6	1.3	2.7	2.5	1.0
Other	40	8.5	0.7	1.4	2.3	0.8
Lot 144 - Enriched 11 mg/dL whole blood						
Bacterial Inhibition Assays	84	11.7	1.4	3.6	2.0	0.9
HPLC	50	13.1	1.0	1.6	1.7	1.1
Tandem Mass Spec	87	13.1	1.4	3.4	2.5	1.0
Other	40	11.4	1.2	2.2	2.3	0.8

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

TABLE 3g. 2001 Quality Control Data
Summaries of Statistical Analyses

METHIONINE (mg Met/dL whole blood)

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 041 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition Assays	40	0.4	0.2	0.3	0.4	1.2
HPLC	30	0.2	0.1	0.1	0.2	0.8
Tandem Mass Spec	48	0.3	0.0	0.1	0.3	0.8
Lot 042 - Enriched 1 mg/dL whole blood						
Bacterial Inhibition Assays	50	1.7	0.5	0.7	0.4	1.2
HPLC	29	1.0	0.1	0.1	0.2	0.8
Tandem Mass Spec	50	1.1	0.3	0.4	0.3	0.8
Lot 043 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition Assays	50	4.0	0.7	1.3	0.4	1.2
HPLC	30	2.8	0.2	0.3	0.2	0.8
Tandem Mass Spec	48	2.6	0.3	0.8	0.3	0.8
Lot 044 - Enriched 6 mg/dL whole blood						
Bacterial Inhibition Assays	48	7.7	1.5	2.7	0.4	1.2
HPLC	30	5.3	0.3	0.4	0.2	0.8
Tandem Mass Spec	50	4.9	0.6	1.5	0.3	0.8

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

METHIONINE (mg Met/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 121 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition Assays	137	0.4	0.2	0.3	0.4	1.1
HPLC	68	0.2	0.1	0.1	0.2	0.8
Tandem Mass Spec	137	0.3	0.2	0.2	0.3	0.8
Lot 122 - Enriched 1 mg/dL whole blood						
Bacterial Inhibition Assays	153	1.4	0.6	0.8	0.4	1.1
HPLC	70	1.0	0.1	0.1	0.2	0.8
Tandem Mass Spec	138	1.0	0.1	0.3	0.3	0.8
Lot 123 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition Assays	168	3.9	1.1	1.7	0.4	1.1
HPLC	69	2.3	0.2	0.2	0.2	0.8
Tandem Mass Spec	139	2.6	0.6	0.9	0.3	0.8
Lot 124 - Enriched 6 mg/dL whole blood						
Bacterial Inhibition Assays	167	6.9	1.2	2.1	0.4	1.1
HPLC	69	4.9	0.4	0.5	0.2	0.8
Tandem Mass Spec	139	5.0	0.7	1.4	0.3	0.8

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

METHIONINE (mg Met/dL whole blood)
- Continued -

Method	N	Mean	Average Within Lab SD	Total SD	Y-Intercept*	Slope
Lot 141 - Nonenriched 0 mg/dL whole blood						
Bacterial Inhibition Assays	90	0.4	0.2	0.4	0.7	1.1
HPLC	38	0.4	0.1	0.1	0.4	0.9
Tandem Mass Spec	98	0.4	0.2	0.2	0.4	0.9
Lot 142 - Enriched 1 mg/dL whole blood						
Bacterial Inhibition Assays	98	1.8	0.5	0.7	0.7	1.1
HPLC	39	1.3	0.2	0.2	0.4	0.9
Tandem Mass Spec	97	1.2	0.2	0.4	0.4	0.9
Lot 143 - Enriched 3 mg/dL whole blood						
Bacterial Inhibition Assays	98	4.4	0.9	1.6	0.7	1.1
HPLC	38	3.3	0.4	0.4	0.4	0.9
Tandem Mass Spec	97	3.0	0.4	0.9	0.4	0.9
Lot 144 - Enriched 6 mg/dL whole blood						
Bacterial Inhibition Assays	96	6.9	1.0	1.7	0.7	1.1
HPLC	39	5.8	0.6	0.6	0.4	0.9
Tandem Mass Spec	98	5.6	0.7	1.4	0.4	0.9

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

NOTES

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